

**STUDIES ON LEAF BLIGHT DISEASE OF
CHILLI (*Capsicum annuum* L.)
CAUSED BY *Alternaria alternata* (FR.) KEISSLER**

Thesis

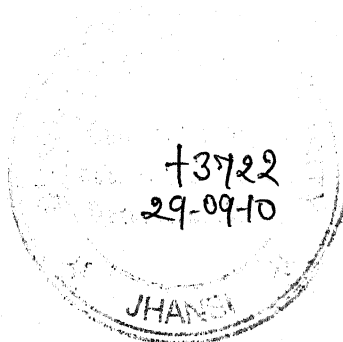
SUBMITTED TO
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IN
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By

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2007



DEDICATED

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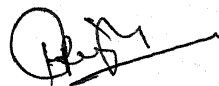
It gives me a great pleasure to certify that the thesis entitled
“Studies on leaf blight disease of chilli (*Capsicum annuum* L.)
caused by *A. Alternata*” is an original piece of work
accomplished by Mr. Harendra Pr. Singh, Under my
supervision for the degree of **Doctor of Philosophy** in the
Department of Botany, Bundelkhand University Campus, Jhansi.

The thesis an embodiment of Mr. Singh's original work and it
fulfils every requirement with regard to its contents as well as his
requisite presence and performance in the department during the
period to complete his research work under my supervision.

(R.K. Agarwal)
Supervisor

DECLARATION

*I hereby declare that the thesis entitled "Studies on leaf blight disease of chilli (*Capsicum annuum* L.) caused by *A. alternata*" being submitted for the degree of Doctor of Philosophy in Botany, Bundelkhand University, Jhansi (UP) is an original piece of research work done by me under the supervision of Dr. R.K. Agarwal. To the best of my knowledge, any part or whole of this thesis has not been submitted for a degree or any other qualification of any university or examining body in India.*



(H.P. Singh)

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(H.P.Singh)

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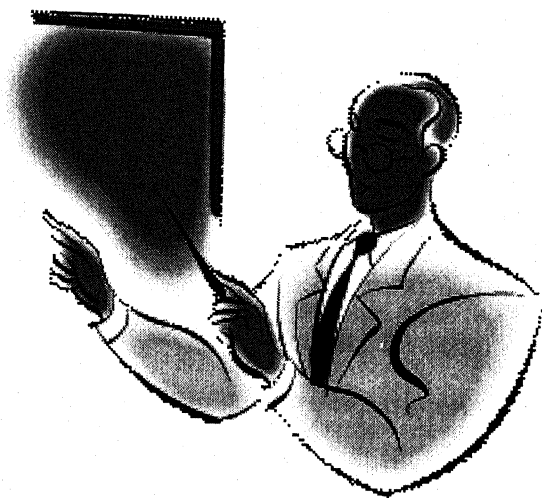
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INTRODUCTION



INTRODUCTION

India is the second largest producer of vegetable next to China. Being predominantly a vegetarian country having common vegetable crop such as *Dolichos lablab* (Bean), *Abelmoschus esculentus* (Okra), *Lycopersicum esculentum* (Tomato), *Solanum melangena* (Brinjal), *Solanum tuberosum* (Potato), *Pisum sativum* (Pea), *Cucurbita moscheta* (Pumpkin), *Momordica chirantia* (Bitter gourd), *Carrisa carandus* (Karonda), *Citrulus vulgaris* (Tinda), *Luffa acutangula* (Ridge gourd), *Brassica oleracea* var. *botrytis* (Cauliflower), *B. oleracea* var. *capitata* (Cabbage), *Raphanus sativus* (Radish), *Allium cepa* (Onion), *Allium sativum* (Garlic), *Capsicum annum* (Chilli), *Brassica repa* (Turnip), *Daucus carota* (Carrot), *Beeta Valgaris* (Beet root), *Ipomoea batatas* (Sweet potato), *Lactuca sativa*, (lettuce), *Spinacea olerace* (Spinach), *Brassica compestris* (Mustard), and *Trigonella foenum graecum* (Methi) etc. India has annual production of about 80 million tonnes of vegetable. The daily per capita consumption is only about 140 g per day. The population is enormously increasing and, therefore, the country would required more than 110 tonnes of vegetable in order to meet the minimum requirment of vegetables per capita per day. Special effort on the intensification of production and supply of vegetable crop are necessary (Majeed and Gowdanage, 1992).

Chillies are the green or dried ripe fruits of *Capsicum annum* L. and sometimes *Capsicum frutescens*. Its specially liked for its pungency, spicy taste, beside the appealing colour it add, to the food. Chillis are one of the most valuable crops of India. Different varieties are grown of vegetables, spices, condiments, sauces and pickles. The crops is grown particularly all over India. Among the most important states only four,

Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu, account for three fourth of the total area however, other states having large areas under chillies are Madhya Pradesh, Punjab and Bihar. However in Uttar Pradesh chillies are grown in almost all the divisions particularly chillies are grown by every farmer either for domestic purpose or commercial status in Banda, Chitrakoot, Hamirpur and Mahoba (Chitrakoot dham/Bundelkand Division) districts (Saini, 2000).

The native place of the chilli is considered to be tropical America, especially Brazil, where it is still found growing in a wild state. In India its introduction is believed to be through the Portuguese in 17th century. At present chillies are indispensable and a common ingredient of south India dietary. It is used throughout the country as condiments.

Chillies belong to the family Solanaceae and genus *Capsicum*. Only four species under cultivation, out of which the cultivation of *C. pendulum* and *C. pubescens*, is restricted to south and central America. The other two, *C. annuum* and *C. frutescens*, are commercially cultivated throughout the world *C. annuum* is the most common cultivated species and all green chillies in the market and most dry chillies belong to this group. All small highly pungent chillies belong to *C. frutescens*. The varieties of chillies are broadly divided into two groups, long pungent types including pickling type and bell shaped non-pungent, mild, thick-fleshed types. The mild types are used as vegetable and commonly known as "Sweet peppers or shimla mirch".

The chillies is a plant of tropical and subtropical regions requiring a warm humid climate. The crop can tolerate extremes of climate better than tomato and brinjal. It also can not withstand long frost and dies at freezing temperature. In general the chilli plants require a temperature of 20-25°C unfavourable temperature and water supply are the basic reasons for bud, blossom and fruit drops. It thrives in area having a moderate

rainfall within the range of 60-120cm. Excessive rainfall however bring about defoliation and rotting of the plants. Chillies can be grown from the sea level upto an altitude of 200 meters. In *Capsicum* fruits development was found to be adversely affected at temperature of 37°C or more. High temperature and low relative humidity at the time of flowering increased the transpiration full resulting in abscission of buds, flowers and small fruits. Higher night temperature was found to be responsible for the higher capsaicin content (Saini, 2000).

Chillies crops is grown on partically all types of soil accept salty land provided the soil is well drained and well areated where the seasons are shorts, sandy and sandy loam soil are prefered. Acidic and alkaline soils are not suitable for chillies and *Capsicum* growing.

The germination of chillies crop is found to be satisfactory upto a salt level at 4000 ppm with a soil pH of 7.6 and E.C., 0.2. Although sweet pepper can grow almost all types of soil, well drined clay loam soil is considered as ideal for cultivation of sweet pepper. On sandy loam soil crop can successfully be grown provided manuring is done heavily and the crop is irrigated properly and timely. The pH was 6.0-6.5 (Saini, 2000).

Chilli is a unique crop among all the spice crops being the only source of capsaicin (Tiwari, 1990). The food value of chilli has been mentioned in the hand book of ICAR, New Delhi (Singh, 1989). The chillies good source of vitamin A. Recently Russion scientists identified vitamin P in grean chillies which they discovered to confirmed protection against damage due to secondary irradiation caused by atomic explosion (Singh, 1989). Where as vitamin A is esential for growth, eye and reproduction. It also help in resistance to infection, increase longevity and decrease senility. Deficiency of vitamin A cause nightblindness, retardation in growth, roughness of skin and formation of stone, in kidney

and Gall bladder. Ascorbic acid and vitamin C are abundant in green chillies which are essential in diet for human beings as they are not synthesized by them. Their deficiency results in unhealthy gums and delay in wound healing. The nutritive value of green chilli have been summarized by saini, 2000.

Table A-The Nutritive value of green chilli as per 100g of edible portion (Saini, 2000).

Nutritive Content	Amocent
Moisture	85.70g
Protein	2.90g
Fat	0.60g
Minerals	1.00g
Fibers	6.80g
Other Carbohydrate	3.00g
Calcium	30.00mg
Magnesium	24.00mg
Riboflavin	0.39mg
Oxalic acid	67.00mg
Vitamin A	292.00IU
Phosphorous	80.00mg
Iron	1.20mg
Sodium	6.50mg
Potassium	217.00mg
Copper	1.55mg
Sulphur	34.00mg
Chlorine	15.00mg
Thiamine	0.19mg
Nicotinic acid	0.90mg
Vitamin C	111.00mg
Energy	29.00cal.

Capsicum annuum fruits are tapering, much longer than broad and very pungent with thick-flesh medicinally the fruit are used in hoarseness and dyspepsia. They are also applied in snakebite and long bite. Being used in almost all kind of foods the fruits are also used as preventive of heart diseases and also as a curative for many rheumatic troubles. The green chilli also contain rutin which has specific medicinal value (Purseglove, 1977). The pungency of chilli is due to an alkaloid capsaicin ($C_{18}H_{27}NO_3$). The active hot principle which is used in pharmaceuticals, cosmetic, food and drinks, (Tiwari, 1990). The red colour in fruits at the ripening stage is due to the pigment capsaicin (Nath, 1969). Chillies are valued throughout the world for pungency. The pungent principle of red pepper consists of a mixture of seven closely related alkyl vanillylamides named as capsaicinoids which are separated by solvent extraction of the dried fruits and the subsequent extraction of the dried fruits and the subsequent removal of the solvent (Tiwari, 1990; Govind rajan, 1985; Maya, 1975).

According to Chamber (2005) Chillies are growing in around 834.3 thousands hectares with annual production of 847.8 thousands MT and productivity of an 1.0 MT/hectares in India. In U.P. chillies are grown in 15516 hectares with a total annual production of 15632 Tonnes and productivity 0.879 M.T./hectares.

India exports chillies and their products to various countries Abudhabi, Australia, Canada, Japan, USA and UK with around 25000 Tonnes costing about Rs. 76 crores (Anonymous, 1992). In India almost all the states cultivate chillies. However Andhra Pradesh, Bihar, Gujarat, Karnataka, Maharashtra, Madhya Pradesh, Punjab, Tamil Nadu, Uttar Pradesh, are the major chilli producers.

The disease was first reported from India in 1937 and later was observed and reported from various chilli growing areas of the world

namely, Argentina, Bangladesh, Brazil, Italy, Mexico, Pakistan, Poland, Romania, U.S.A. and former USSR.

Udit Narain, *et al.*, (2000) reported that *A. alternata* caused losses a wide range of 5-85% in chilli in India severe seed infection resulted in great losses in Italy destroying seed teguments and embryo in Nigeria, 40-45% and 25-35% losses in wet and dry season, respectively due to fungal spoilage. The losses in hot and sweet capsicum variety was estimated to be 9.6 to 21.6% and 3.7 to 79.0%, respectively in Illinois, U.S.A. severe bronzing of sweet pepper caused by *A. alternata* in the north of Balkan mountain of Bulgaria with 60-70% losses were recorded in Popovska and Tergovishte regions. Losses of 30-40% fruits due to the infection of *A. Alternata* and in Mexico, 80-90% losses due to internal infection of *A. species* were recorded. An estimated losses of 75-85% has been observed at Kanpur, U.P. and upto 8.32% loss in Assam and Maharashtra and disease incidence ranged from 8.3-21.6% in M.P., the disease caused a loss of 30.76% of the produced.

Chilli is an important vegetable crop, which suffers from several diseases such as seedling blight caused by *Rhizoctonia spp.*, Damping off caused by *Pythium spp.*, and *Phytophthora spp.*, wilt caused by *Fusarium spp.*, leaf blight is caused by *Alternaria solani*, leaf spot and fruit rot caused by *Alternaria alternata*, Anthracnose and Dieback caused by *Colletotrichum capsici*, Bacterial leaf spot caused by *Xanthomonas spp.*, Mosaic disease caused by virus, as well as leaf curl is also caused by virus (Oelke and Bosland, 2001).

It is affected by 750 pathogen of different origins (44) which have been reported from different part of the world, but only a few cause considerable damage to this crop. Among the fungal diseases, leaf spot and fruit rot due to *Alternaria alternata* (Fr.) Keissler cause considerable qualitative losses to the crop. *Alternaria altrnata*, the causative agent of

this disease was considered to be the pathogen of minor importance in the past, but now a days, it is appearing in server form and is posing a major problem in chilli growing regions of the country the pathogen has been reported to cause seed, seedling leaf and fruit diseases a part from post harvest decay of fruits and seed. (Udit Narain and Bhale, 2000).

The chilli crop suffers due to number of fangal, bacterial and viral diseases which under its production on into stake (Agriose, 1988; Mukherji and Bhasin, 1986; Singh, 1983). Among the various fungal diseases leaf spot fruit rot incited by *Alternaria alternata* is becoming a limiting factor and posing a major problems in Chitrakoot dham division of U.P. (Verma and Bhale, 1989, 1983). The pathogen has been reported to cause seed, seedling leaf and fruit diseases (Sreekantiah *et al.*, 1973; Edward and Srivastava, 1954). Post harvest decay of fruits and seed has also been recorded due to this pathogen (Spalding and King, 1981; Mathur and Agnihotri, 1961; Leyendecker, 1954a).

Leaf blight and fruit rot of chilli caused by *Alternaria alternata* (Fr) Keissler, mycelium consist of septate, branched, light brown hyphe which become darker with age. The hyphe are at first intercellular, later penetrating into the cells of the invaded tissues. Conidiophore emerged through the stomata from the dead center of the leaf spot. These are 50-90 x 9-10 μ , dark coloured and borne single in culture or they from chains of conidia (Chains of 2-3 conidia only); conidia are beaked, muri form, dark coloured and 120-296 x 12-20 μ . There are both transverse and longitudinal septa in the mature conidia. *Alternaria alternata* produces a non-specific toxin known as alternaric acid. The role of this acid in disease is doubtful (Mehrotra, 1980).

The Symtoms of diseases are observed from seedling to maturity stage of the crop and even early blight symptoms are noticed in nursury stage (11). Necrosis of tender twigs also accurs trom tips downward (23).

On the leaves and particularly on fruits. Initially small scattered brown coloured necrotic spots that gradually enlarge in size are formed. The mature spots are irregular to circular in shape and upto 8-10 mm in diameter. These spots are accompanied by narrow chlorotic margin. Finally, The spots coalesce, which result in withering and shedding of leaves. Due to humid conditions prevailing at the ground level, lower leaves are affected first and the infection spread to upper leaves later on (7, 56). In the beginning, small, blackish brown, circular to elongated, watersoaked depressed lesion are formed on the pericarp of fruits, which lead the rotting of fruits in later stage (4, 16, 54). Sometime the characteristic lesion are not seen on green fruits and fruits rot is visible at maturity stage (61, 70). In the form of minute, grayish green lesion are round at the blossom end, which stage (51) and lead the blighting and extensive rotting of the respectable, longitudinal cracks on the pericarp can also be seen which often coalesce and ultimately cover the appreciable area of the fruits with abundant sporulation of the fungus (6.0). A part from the symptoms on leaves, twigs and fruits caused by *A. alternata* also incites internal infection of fruits as "Internal mould" during post harvest handling, transit and storage (Udit Narain *et al*, 2000).

The pathogen survive in the field in plant debris and the secondary infection take place by wind borne conidia. *Alternaria alternata* has a wide host range including the other members of the family Solanaceae. The infected seed obtained from infected fruits serve as basic source of Inoculum while the secondary infection take place through the inoculum derived from the sporulation on the infected portion. The role of rains and wind is the most important in the development of the disease in the field. Seed play an important and vital role in the production of healthy crop. They are known to carry pathogen which cause heavy losses. Chilli crop suffers due to number of diseases from seed rot,

seedling decay to maladies of adult plant. Many of the diseases of chilli are seed borne. Seed borne nature of *Alternaria alternata* in case of *Capsicum annuum* has been demonstrated and reported by several worker. Thus leaf spot and fruit rot disease is seed, soil and air borne in nature and there is also seed transmission of *A. alternata* in chilli (Udit Narain and Bhale, 2000).

The economic management of plant diseases is a perpetual process involving conventional and non-conventional method of chemical control, disease forecasting and evaluation of disease resistant varieties in order to prevent the crop from poor yield per unit area in the country. The foliar disease are known is cause reduction in the yield upto about 30% among the agent causing disease are fungi, bacteria and virus that hamper the successful cultivation of chilli. Therefore, suitable protection and production technology must be evolved as well as use of chemicals and genetic manipulations along with cultural practicess for disease control must be adopted (Droby *et al*, 1998; Agranovsky, 1993).

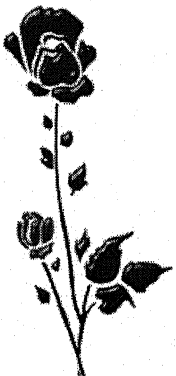
Chilli crop has been observed to suffer due to pre and post emergence rots. Leaf spot, fruit rot, and tender tip drying incited by *Alternaria alternata*. Heavy losses are incured in the nursery stage and fruit stage. Attempts have been made by several workers to find suitable measures for the management of the disease (12, 15, 22). Evalation of germplasm and chemical control methods are the general and well adopted approaches. Fungitoxicity of extract of 10 botanical family and plant products was recorded unders pot conditions. Two spray of the leaf extract (10%) of *Aegle marmelos* (Bel) combined with 0.01ml nickel sulphate at 100 and 115 days after sowing significantly reduced the disease intensity (61). A simple method has been adopted and suggested for reducing the losses in chilli caused by *Alternaria alternata* by storing

fruits at 16-26°C and taking care during picking of the fruits (36) (Udit narain and Bhale, 2000).

Considering the importance of the disease in causing significant annual yield loss, present studies of leaf and fruit rot of chilli caused by *A. Alternata* problem in growing areas of the our country, non availability of suitable management practices and gaps in our knowledge about this disease, the present research work has been undertaken with following objective:-

1. Survey and collection of the diseased material from the different locations in U.P., and evaluate the losses of diseases.
2. To isolate and identification of the established pathogens.
3. To screen available cultivars and varieties against the disease.
4. To Study the morphological and physiological character of different isolate of the pathogen obtained from different locations.
5. Role of seed in transmission of the pathogen in causing the disease.
6. To study the mycoflora associated with *Alternaria* infected seed.
7. To develop techinque for the direct detection of *Alternaria alternata* associated with chilli seed.
8. To study the effect of fungal infection on the nutritive value of chilli fruits, especially, capsaicin and vitamin C.
9. To make investigation on the epidemiology of the disease.
10. To evaluate various agrochemicals for the management of alternaria leaf spot and fruit rot *in vivo* and *in vitro* conditions.

REVIEW OF LITERATURE



REVIEW OF LITERATURE

Chilli (*Capsicum annum* L.) is a unique crop among all the spices and condiments being the only source of capsaicin. It belongs to family Solanaceae having chromosome number $2n=24$. The place of origin of chilli is Mexico and Guate Mala (Singh, 1989). It was introduced by Portugese in India. The crop suffers due to a number of diseases (Chupp and Sherf, 1960; Mehrotra, 1980; Singh, 1987).

According to Mukherji and Bhasin (1986), about 47 organisms have been found responsible for causing diseases in chilli, of which the following are the major fungal diseases.

Leaf spot, furit rot and dieback (*Alternaria alternata*), leaf spots (*Botryodiplodia theobrome*), leaf spots (*Cercospora, capsici*), fruit rot (*Chaetomium globossum*), flower and fruit rot (*Choanephora cucurbitarum*), fruit rot (*Cladosporium oxysporum*), fruit rot and anthracnose (*Colletotrichum capsici*, *C. inamadari*, *C. piperatum*), leaf spot and fruit rot (*Corynespora cassicola*), fruit rot (*Curvularia lunata*), leaf spot (*Curvularia ovoida*), fruit rot (*Diplodia natalensis*), fruit rot (*Drechslera tetramera*), seed infection, fruit rot (*Fusarium semitectum* and *Fusarium spp.*), Anthracnose (*Gloeosporium piperatum*), fruit rot and leaf spot (*Glomerella cingulate*), leaf spots and fruit rot (*Helminthosporium capsicicola*), powdery mildew (*Leveillula taurcia*, *oidiopsis sicula*, *O. taurica*), leaf blight (*Periconia byssoides*), fruit infection (*Pestalotiopsis palmarum*), fruit rot (*Phoma capsici*), leaf spot (*Phyllosticta capsici*), leaf blight, fruit rot (*Phytophthora nicotiane* var. *nicotiane*), fruit rot (*Rhizoctonia bataticola*), stem and root rot (*Sclerotinia sclerotiorum*) and fruit rot (*Sclerotium rolfsii*).

In the present investigation, the review of literature on chilli leaf spot and fruit rot caused by *Alternaria alternata*. (Fr.) Keissler revealed that so far very little work has been done, however, the occurrence report of the disease has been made from many places. The reason being the very restricted distribution of the disease and so far the disease was not considered as potential problem. In the present review of literature, effort has been made to cite references of work done on *Alternaria alternata*. In majority of the aspects covered in the present investigation, no references regarding *A. alternata* on chilli were available, hence references of work done by different workers on this pathogen on other crops have been cited to know about the host pathogen relationship.

1. Geographical distribution and occurrence

The disease has been observed and reported from various chilli growing areas of the world causing seed and seedling infection, leaf spots, fruit rot and die back. The pathogen has also been observed in fruits during post harvest handling.

The disease has been reported from New Mexico (*Leyendecker*, 1954a), Italy (*Verneau*, 1954; *Treggi and Bertini*, 1955; *Sibilia*, 1957), Nigeria and Brazil (*Uma*, 1981; *Adisa*, 1985), USA (*Courter et al.*, 1965; *Cuebral and Shurtleff*, 1965; *Miller et al.*, 1984), Bangladesh (*Alam et al.*, 1981; *Mridha and Siddiqui*, 1989), Romania (*Anonymous*, 1957; *Tutunaru and Raicu*, 1978), Iraq (*Shawkat et al.*, 1978), Pakistan (*Perwaize et al.*, 1968; *Kamal and Tahiruddin*, 1970; *Sultana et al.*, 1988), former USSR (*Melikova*, 1960), Poland (*Kharkova et al.*, 1974), Mexico (*Brauer and Richardson*, 1957), Argentina (*Walter et al.*, 1974), and Armenia (*Babayan and Shakhnubaryan*, 1976).

The disease has also been recorded in India. Reports from various parts include Rajasthan (Mathur and Agnihotri, 1961). Uttar Pradesh (Edward and Shrivastava, 1954; Singh and Tandon, 1967), Punjab (Thind and Jhooty, 1985; Bansal *et al.*, 1987), Madhya Pradesh (Jain *et al.*, 1973; Jharia *et al.*, 1977; Hasiya, 1987; Verma and Bhale, 1989; Padagnur and Naik, 1991), Maharashtra (Dhawale and Kodmelwar, 1978; Mali and Joi, 1985), Tamil Nadu (Pillayarswamy *et al.*, 1973; Sujatha Bai *et al.*, 1993a, 1993b), Haryana (Chauhan and Duhan, 1986), Andhra Pradesh (Manoharachary and Padmavati, 1976).

2. Symptomatology

Symptoms incited by the fungus have been studied, observed and described by many workers. In literature, more often the disease is referred as fruit rot and blight.

A small black brown circular spot is formed on the pericarp of the chilli fruits turning red, then brown which spreads in the direction of long axis. As the infection progresses the spots get either diffused and brown dirty grey. Badly infected fruits turn straw coloured from normal red. In case of die back necrosis of tender twigs occurs from tip downwards. Target board type typical symptoms are also common in leaf infection.

Sujatha Bai *et al.* (1993a) reported the fruit rot at maturity stage. The diseased fruits were black and rotten, however, they did not find the infection on green fruits and leaves, The rotting of fruits was also recorded by Sibilia (1957); Melikova (1959, 1960); Courter and Shurtleff (1965); Alam *et al.*, (1981) observed the infection as black spots on fruits. Brauer and Recharadson (1957) recorded the early blight symptoms in nursery stage. Queberal and Shurtleff (1965) also observed the fruit rot infection, however, primary infection at the blossom end was recorded as

minute grayish green lesions which turned darker and became wrinkled while tutunaru and Raicu (1970) observed blight and extensive rotting of the receptacle. Severe seed infection in the fruits was also recorded by them. Uma (1981) observed the association of the fungus on ripe matured fruits of chilli. Longitudinal cracks in the pericarp of chilli fruits were recorded by shawkat *et al.*, (1978) which often coalesced and ultimately covered the appreciable area of the fruits. Sreekantiah *et al.*, (1973) recorded severe leaf spot and fruit rot. Edward and Shrivastava (1954) observed the die back symptoms.

Seed rot and seedling decay symptoms have been reported by Suryanarayana and Bhombe (1961); Harne and Nena (1967); walter *et al.* (1974) and suryanarayana (1978) A part from these symptoms, the fungus *Alternaria alternata* also incited interanal infection of fruits as mould during post harvest handling. Transit and storage (Leyendecker, 1954a; Mathur and Agnihorti, 1961; spalding and king 1981; Miller *et al.*, 1984 and Adisa 1985).

3. Nature and Extent of deamage

The pathogen survives in the field in plant debris and the secondary infection takes place by wind-borne conidia. The pathogen has a wide host range including the other members of the family Solanaceae. Seeds from badly diseased fruits also carry the primary inoculum. Even apparently healthy (spotless) fruits may contain the infected rusty brown seeds. Therefore, the disease is seed, soil and airborne in nature (Mehrotra, 1980; Singh, 1983; Singh, 1987; Agrios, 1988).

3.1 Seed-borne Nature

The seed-borne nature of *Alternaria alternata* in case of *Capsicum annum* has been demonstrated and reported by several workers

(Suryanaryana and Bhombe, 1961; Rout and Rath, 1972; Manoharachari and Padmavati, 1976; Dhawale and Kadmelwar, 1978; Suryanarayana, 1978; Shawakat *et al.*, 1978; Advar *et al.*, 1987; Agrawal and Sinclair, 1987; Mridha and Siddiqui, 1989; Hashmi, 1989).

The seed-borne nature of *Alternaria sp.* has been well documented and demonstrated on other crops. For example, *Alternaria carthami* with safflower seeds (Irwin, 1976; Varma, 1987), *A. sesamicola* with sesame seeds (Singh *et al.*, 1990), *A. brassicicola* with mustards seeds (Domsch, 1957), *A. loogipus* with tobacco (Heursel, 1961). *A. helianthi* with sunflower (singh *et al.*, 1977), *A. longissima* with sesamum (Yu *et al.*, 1982). *A. alternata* with chickpea (Haware *et al.*, 1986, Bhargava *et al.*, 1992), *Alternaria sp.* With mustard (Shah and Jain, 1981).

3.2. Extent of damage

Very limited attempts have been made to estimate the extent of damage due to *Alternaria alternata* in chillies. Mathur and Agnihotri (1961) observed a wide range of damage due to fungal attack in chillies. They observed 5 to 85 per cent losses due to *A. tenuis* at Udaipur. Verneau (1954) recorded the losses from 25 to 30 per cent. Severe seed infection resulted in great losses in Italy destroying seed teguments and embryo. Adisa (1985) observed 40 to 45 per cent and 25 to 35 per cent losses in wet and dry seasons due to fungal spoilage in Nigeria. Quberal and Shurtleff (1965) estimated the losses in hot and sweet *Capsicum* varieties and reported 9.6 to 21.6 per cent and 3.7 to 79.0 per cent losses respectively in Illinois, USA. Kovachevski and Markov (1959) observed severe bronzing of egg plant, sweet pepper and tobacco caused by *Alternaria sp.* in the north of Balkan mountains of Bulgaria. They reported 60-70 per cent losses in Popovska and Tergovishter regions of Bulgaria. Singh (1987) reported the losses ranging from 19.0 to 47.89 per cent, while Sanz (1970) observed losses of 30 to 40 per cent fruits due to

the infection of *A. tenuis*. In New Mexico, 80-90% losses due to internal infection of *Alternaria* sp. Were recorded (Leyendecker, 1954b).

Reduced seed germination and seed rot due to *Alternaria* sp. has been observed by Adiver *et al.*, (1987) and Dhawale and Kodmelwar (1978).

4. Pathogen :

4.1 Nomenclature and History

The fungus *Alternaria tenuis* was originally described by Nees in 1816. Angell (1929) and Wiltshire (1933) suggested that the species of *Alternaria* may be placed in two categories:

- (A) Species forming chains of spores with a relatively short beak.
- (B) Species seldom forming conidial chains and in which the conidia have long filiform beak.

Later on, in 1945, Neergaard placed the species of *Alternaria* in three different sections:

- Section-1. Longicatenate, Section-2. Brevicatenate,
- Section-3. Noncatenate

Alternaria alternata (Fries) Keissler (= *Alternaria tenuis* (Ness)) is as explained by Simon (1967), the correct name of the type species *Alternaria*. However, *A. tenuis* is the commonly used name among the scientists for the synonym of *A. alternata* which are generally characterized by long conidial chain and marked by polymorphic conidia.

Alternaria longipes (Ell. And Ev.) Mason was suggested by Mason (1928) with characters conidiophore effuse, torulose, 40-70 μ long, 3-4 μ wide conidia obclavate, mostly 5-6 septate, muriform 40-50 μ long, 15-20 μ wide, produced in chains. Lucos (1971) suggested that the name *A. longipes* is not needed and should be placed in synonym with *A. tenuis*

for which, according to International Rules for Nomenclature the valid name is *Alternaria alternata*.

4.2 Cultural and Morphological characters

The morphological and cultural characters of *A. alternata* isolated from various hosts have been described by several workers (Gupta and Das, 1964; Rao, 1965; Aulakh, 1969; Mukhopadhyay, 1969; Dhanraj, 1970; Gupta, 1970; Singh *et al.*, 1980; Gupta and Karwasra, 1983, Singh and Suhag, 1983; Haware *et al.*, 1986).

Singh *et al.*, (1977) reported that on seed colony of *A. tenuis* was amphigenous, wooly, dark brown having long chains, conidiophores arise singly or in groups, simple, light to medium brown, geniculate, 13-163 x 3.5 μ . Conidia polymorphous, short to long, obclavate. One to eight transverse septa with longitudinal septa, 10-58-x7-19 μ . Total length of conidia 10-71 μ .

Haware *et al.*, (1986) described the cultural characters of *A. alternata*. According to them, the colony on PDA is light grey, later black and ultimately olivaceous black. Conidiophore 40-50 μ long and 2-6 μ thick with several conidial scars. Conidia in chains, obclavate with short conical beak, 10-18x 20-65 μ with single beak.

4.3 Pathogenicity:

Pathogenicity is the capability of a pathogen to cause disease. *Alternaria* has a wide host range and exhibit different symptoms on plant parts. It has been found pathogenic to lentil (Gupta and Das, 1964), pea and cowpea (Rao, 1965), mungbean (Gupta, 1970), barley (Dhanraj, 1970). Pear (Taylor, 1970), groundnut (Aulakh, 1969), tomato (Singh and Suhag, 1983), mustard, radish, turnip, cabbage (Changari and Weber, 1963), sunflower (Chowdhury, 1944), sesame (Yu *et al.*, 1982),

sunflower (Singh *et al.*, 1977), Chickpea (Haware *et al.*, 1986, Bhargava *et al.*, 1992), wheat (Hyde and Galleymore, 1951).

Changari and Weber (1963) proved the pathogenicity of *Alternaria* spp. by soil infestation method for cruciferous seeds while Babadoost and Gabrison (1979) used seeding inoculation method. Pathogenicity of *A. carthami* was studied by Singh and Chand (1982) and Mc Rae *et al.*, (1984). Yu *et al.*, (1982) proved the pathogenicity of *A. sesame*, *A. sesamicola*, *A. Tenuis* and *A. longissima* by spore suspension spray method. Singh *et al.*, (1977) studied the pathogenicity of *A. tenuis* by seed infestation method while Hyde and Gallemore (1951) studied the pathogenicity in wheat grains by seed inoculation. Bhargava *et al.*, (1982) proved the pathogenicity of *Alternaria alternata* by spore suspension spray method, while Khare (1979) also suggested this method for *A. porri*. Sultana *et al.*, (1988) demonstrated that wounded and injured chilli fruits had greater infection due to *A. alternata*.

A good account of the pathogenicity and host range of *Alternaria* sp. On solanaceous crops has been dealt by Agrios (1988) and Singh (1987).

5. Detection of mycoflora associated with chilli seeds

Studies on the association of mycoflora with chilli seeds have been carried out by several workers (Mishra, 1963; Rout and Rath, 1972; Manoharachari and Padmavati, 1976; Chourasia, 1976; Siddiqui *et al.*, 1977; adiver *et al.*, 1987; Hashmi, 1989;).

Seeds play an important and vital role in the production of healthy crop. They are known to carry pathogens which cause heavy yield losses (Neergaard, 1977; Agarwal and Sinclair, 1987). Chilli crop suffers due to a number of diseases from seed rot, seedling decay to maladies of adult plant (chupp and Sherf, 1960). Many of the diseases of chilli are seed-

borne (Nobel and Rechardson, 1968; Suryanarayana, 1978; Padaganur and Naik, 1991).

Suryanarayana and Bhombe (1961) isolated species of *Alternaria*, *colletotrichum*, *curvularia*, *Fusarium*, *Penicillium*, *Helminthosporium* and *Phomopsis* from chilli seeds. Ram Nath and Lambet (1971) reported *Drechslera hawaiiensis* on chilli seeds. Walter *et al.*, (1974) observed *Alternaria* sp., *Drechslera sorokiniana*, *D. tetramera*, *Fusarium equisetii*, *F. moniliforme*, *F. semitettum*, *F. solani*, *Myrothecium verrucaria*, *Phoma* sp. and *Colletotrichum capsici*. Babayan and Shakhunbaryan (1970) observed *Fusarium oxysporum*, *Verticillium alboatrum* associated with chilli seeds. Mishra (1963), Harne and Nema (1967) recorded *Aspergillus*, *Rhizopus*, *Curvularia*, *Rhizoctonia*, *Alternaria*, *Rhizopus nigricans*, *Alternaria tenuis*, *C. lunata* from chilli seeds. Seed-borne nature of *Alternaria alternata* in *Capsicum annuum* has also been reported by Chourasia (1976). Mishra (1963), Siddiqui *et al.*, (1977), Hashmi (1989), Mridha and siddiqui (1989), Sultana *et al.*, (1988).

5.1 Methods for the detection of mycoflora

Several methods have been standardized and recommended as standard seed testing methods for the detection of seedborne fungi by the International seed Testing Association. Blotter method was first adopted by Doyar (1938) in seed health testing which is still in use and most convenient. Agar-plate method was developed by Musket and Celhoun (1948) incubating flax seeds on malt extract at ulster. The medium is now replaced by potato dextrose agar. The Blotter method has been modified in several ways. At seed testing stations, rolled paper towel method (Ragdoll method) is used for germination test as well as for seed health testing.

Neegaard (1973) introduced the use of 2, 4-D salt in blotter method. Hagborg *et al.* (1950) first used 2, 4-D in agar medium for

detecting the seed-borne fungi. Deep freezing blotter method was developed at Wageningen. The Netherlands and was first used in seed health testing by Limonard (1968). This method checks the germination of seed and thus facilitates in recording observations.

6. Disease Cycle

Disease cycle of *Alternaria alternata* causing leaf spot, fruit rot and tender twig drying of chilli has not been fully studied, however, Agrios (1988) has attempted to compile information on this aspect. Similarly, there is no clear information available for the transmission of the pathogen from seed to plant and plant to seed.

According to Agrios (1988), the alternaria diseases are among the widespread problems of many kinds of plants which primarily affect the leaves, stem, flower and fruits. Lower senescent leaves are usually attacked first and progress upward and makes affected leaves go into a yellowish senescence and either dry up or droop off. Seedlings are attacked by the fungus and kill the plants. On the infected plant parts, fungus produces abundant spores which are usually carried by air currents. Plant pathogenic species of *Alternaria* overwinter as mycelium in infected plant debris and as mycelium of spores in or on seeds. If the fungus is carried with the seeds, it may attack seedlings and after emergence it causes damping off or stem lesions. More frequently, the spores that are produced abundantly especially in heavy dews and frequent rains, are blown which settle on plant parts and infect plant. The germinating spores penetrate susceptible tissues directly or through wounds and soon produce new conidia that are further spread by wind, splashing rains or tools. This cycle is repeated again. The information for chilli is lacking on

this aspect, however, few scanty informations are available on other crops.

Association of *Alternaria alternata* with chilli seeds has been well documented by several workers (Mishra, 1963; Harne and Nama, 1967; Rout and Rath, 1972; Manoharachari and Padmavati, 1976; Suryanarayana and Bhombe, 1976; Suryanarayana, 1978; Adiver *et al.*, 1987). Although no histopathological studies have been made for *Alternaria alternata* (chilli isolate) but studies are conducted with sunflower and sesame. These studies indicate the deep penetration of *Alternaria alternata*. Singh *et al.*, (1977), Singh *et al.*, (1980) and Singh *et al.*, (1983) have demonstrated the extra and intra embryonic nature of the infection of *Alternaria sp.* They have clarified that the dormant mycelium remains ecto as well as endophytically and in all the layers of pericarp and testa. Aggregation of the mycelium beneath the cuticle has also been demonstrated indicating the direct and indirect penetration by the fungus (Singh *et al.*, 1980).

Seed to seedling transmission of *A. sesamicola* was confirmed by Singh *et al.*, (1983). They reported that in growing-on tests seeds which failed to germinate were found to be covered with conidia of *A. sesamicola*. The infected and semi-dead seedlings yielded the fungus upon isolation on PDA exhibiting the systemic nature and systemic seed transmission of *Alternaria sp.* Pre, and post-emergence mortality due to the fungus was also recorded indicating the role of seeds in causing the disease by *Alternaria sp.* (Singh *et al.*, 1983). Khare (1979) also recorded the penetration of onion leaves by *Alternaria porri* and infection of mesophyll and parenchymatous cells which later on collapse and form the cavity which is often filled by mycelium. Sultana *et al.*, (1988) reported the seed transmission of *Alternaria alternata* on *Capsicum annuum* in Sindh, Pakistan.

There are very few reports on the mode and period of survival of *Alternaria alternata*. Bhargava *et al.*, (1992) observed that the fungus can survive saprophytically on plant debris left in the field. They found that the inoculum gets reduced to 10% on seed and 30% in debris upto the next sowing time in chickpea, whereas Laszlo (1969) reported that *Alternaria tenuis* could exist saprophytically for a long time and retain its pathogenicity. Favourable conditions can then create an epidemic which depends on the conditions and development of the plant and on the seasonal factors. Suhag and Singh (1984) studied the survival of the pathogen on seed and debris of radish by plating the infected portions on the medium each month. They observed that recovery of the pathogen from seed was reduced to about 10% by the next sowing time. The importance of the role of seed as primary source of inoculum was realized (Suhag and Singh, 1984).

Hussain (1960) found that *Alternaria porri* the incitant of onion blotch overwinters in debris under New York conditions and similar results were obtained by Pandotra (1965) in Punjab, India. Neshsev *et al.*, (1975) found that *Alternaria porri* overwinters on plant debris as spores and mycelium, while Basu (1971) recorded the survival of *Alternaria porri* f. *sp. solani* in the form of chlamydospores for seven months. The chlamydospores caused primary infection for next crop and could survive even without host.

The number of spores produced by a pathogen on its host reflects the pathogenicity and it is the sum of the effect of all the components of resistance mechanism in the host (Agrios, 1988), little is known about the sporulation of *Alteraria alternata* causing disease of chilli on media and host. Rotem (1978) observed the increased sporulation of *Alternaria porri* on leaves sprayed with water and positive effect of light.

The dispersal of spores usually takes place by air currents and the aerobiological studies of *Alternaria sp.* have been undertaken by several workers (Alicubsan *et al.*, 1959; Hussain, 1960; Bhargava *et al.*, 1992; Khare, 1979; Meredith, 1966; Bhargava and Khare, 1988a).

The infected seeds obtained from infected fruits serve as basic source of inoculum while the secondary infection takes place through the inoculum derived from the sporulation on the infected portion. The role of rains and wind is the most important in the development of the disease (Alicubsan *et al.*, 1959; Rotem, 1963; Khare, 1979; Singh, 1987; Bhargava and Khare, 1988 a, b; Bhargava *et al.*, 1992).

7. Factors affecting growth and sporulation of *Alternaria sp.*:

7.1 Effect of media

It is essential to have suitable medium for maximum growth and sporulation of a fungus. Bhargava *et al.*, (1992) evaluated eight media for *Alternaria alternata* (Chickpea isolate) and observed that chickpea seed extract medium and potato dextrose agar were the best, while Fahim (1966) observed PDA to be the best medium for the fungus. On the contrary, Bock (1964) found that onion agar was better than PDA. Mohanty *et al.* (1981) reported that Richard's medium was the best for *A. carthami*, whereas Pero and Main (1970) found the favourable growth of *A. tenuis* on autoclaved rice grain supplemented with yeast extract and Czapek's broth. Siddaramaiah and Hegde (1984) reported good growth of *A. alternata* on PDA. Similar trend was recorded by Singh and Khanna (1969) and Hasan (1970). According to Mehta and Prasad (1976), oat meal agar was the best solid and Richard's the best liquid medium for *Alternaria sp.* isolate of sesame.

7.2. Effect of temperature and pH

Temperature and hydrogen ion concentration are the other important factors which influence the growth as well as sporulation of a fungus. Bhargava *et al.*, (1992) pointed out maximum colony growth and sporulation at 25⁰C and maximum colony growth and pH 6.0 but maximum sporulation at pH 5.0 for *A. alternata*. Similar observations for temperature requirement were recorded by Fahim (1966). Arya and Prasad (1952) indicated that pH 3.0 to 8.5 is suitable for the growth of *Alternaria sp.* (linseed isolate) while Mohanty *et al.*, (1981) recorded the best growth at pH 6.0 Samual *et al.*, (1971) recorded good mycelial growth and sporulation between 4.0 to 8.0 pH and pH 5.0 was optimum for mycelial growth and 24-26⁰C for *A. alternata*. Hasan (1970) reported best temperature 15 to 25⁰C (optimum 25⁰C) and pH 2.7 to 8.0 (Optimum 5.4) while Jamaluddin and Tondon (1975) reported pH 7.0 best for mycelial growth.

7.3 Effect on spore germination

Spore germination is a pre-requisite for infection of host by a fungal pathogen. According to Wolf and Wolf (1947) spore germination is influenced by the two distinct group of factors viz. heredity or internal and environmental or external factors. Among the environmental factors, moisture, temperature, pH, nutrient, aeration and presence of stimulating or inhibitory substances have been reported to exert a profound influence on this process.

Pawar and Patel (1957) observed that spore germination of *Alternaria spp.* was the best at 25 to 30⁰C while Bock (1964) found a broad range from 25 to 30⁰C and Bock (1964) also recorded a range from 21 to 30⁰C for spore germination. Chowdhury (1944) concluded that 25⁰C and 30⁰C was favourable for spore germination of *A. carthami* at pH 6.7

Similar observations were made by Verma (1987) on *A. carthami*. A good account of work on spore germination of *A. sesame* has been presented by Berry (1960); Samuel *et al.*, (1971); Mohapatra *et al.*, (1978) and Rajpurohit and Prasas (1982),

7.4 Effect of seed exudates

During seed germination several biochemical changes occur and several chemicals are released as such or as metabolites in the form of exudates. These exudations may enhance germination of spores (Schroth and synder, 1961; Youseff and Youseff, 1971; Naim *et al.*, 1976). On the other hand, they may be antimicrobial in nature and may either inhibit the spore germination or suppress the fungal growth (Agrawal and Khare, 1974; Kraft, 1974; Shukla, 1974).

8. Factors affecting disease development

Temperature, humid weather with high atmospheric humidity, precipitation in the form of rains, dense mist, fog and dew are of prime importance in boosting the development of a disease (Aust, 1986). Relative humidity in the microclimate of crop canopy also plays an important role (Bhargava and Khare, 1988 a, b).

Higher incidence of *Alternaria alternata* in chilli fruits was recorded at 26°C by Mathur and Agnihotri (1961) while Verneau (1954) found 30°C favourable for maximum rotting of stored chilli fruits. Adisa (1985) recorded the influence of dry and wet seasons on the spoilage of chilli and found the higher degree of infection in wet season.

Importance of environmental factors has been realized by other workers in onion blotch caused by *Alternaria porri* (Bock, 1964; Pandotra, 1964; Boelema and Ehlers, 1967).

According to Bock (1964), post infection relative humidity plays an important role in the development of symptoms. High relative humidity favoured the appearance of the purple blotch symptoms caused by *Alternaria porri*. Pandotra (1964) is of the opinion that the severity of the disease depends on the favorable environmental conditions. Treggi and Rainaldi (1966) found that the temperature had a greater effect than relative humidity on the incidence of *Alternaria sp.* on chilli and tomato. However, Bock (1964) noted that the lesions are produced over a wide range of temperature.

Alternaria sp. infection in onion was favoured by wet weather and temperature 21-30°C (Boelema and Ehlers, 1967). Meredith (1966) concluded that wind velocity, dry weather with minimum humidity and subsequent vapour pressure in morning hours induces hygroscopic movements in the conidial apparatus which weakens the attachment of the conidia of *Alternaria* and this leads to easy dispersal. Rotem (1978) observed the higher number of lesions on onion leaves sprayed with water indicating the positive role of humidity in the development of the disease caused by *Alternaria sp.* Bhargava *et al.* (1988) also concluded that relative humidity and congenial temperature are the major factors for the development of blight in chickpea incited by *Alternaria alternata*. Similar observations were made by Suhag *et al.*, (1985) while working with leaf blight of radish caused by *Alternaria alternata*.

The role of temperature in blight of safflower caused by *Alternaria carthami* has been discussed by Mc Rac *et al.*, (1984) and Fahim (1966).

The high humidity is also a major factor for the development of the disease caused by *Alternaria sesame* (Mendez, 1940; Mohanty and Behera, 1958; Mehta and Prasad, 1976).

9. Toxin production

The concept that plant pathogens cause diseases by producing toxic substances dates about a century back. Evidence for its general validity, however, has been accumulated in recent years (Owens, 1969; Ciegler *et al.*, 1971). In many plant diseases host susceptible interactions are associated with the synthesis of host invading substances like toxins and other metabolites. Effect and mode of action of such substances has been demonstrated by Bram (1955); Wheeler and Luke (1963), Pringle and Scheffer (1964); Sadashivan and Kalyanasundaram (1965), and Sadashivan (1969).

A toxin can be defined as a microbial metabolite excreted or released by lysed cells which in very low concentration is directly toxic to cells of the susceptible (Gareth, 1987).

Alternaria tenuis (= *A. alternata*) produces tentoxin which causes chlorosis. Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protein (chloroplast-coupling factor) involved in energy transfer into chloroplasts and also inhibits the light dependent phosphorylation of ADP to ATP. Both the inactivation of the protein and inhibition of photophosphorylation is recorded. The toxin inhibits the activity of polyphenyl oxidase enzyme involved in several resistance mechanisms of plant. The tentoxin produced by *Alternaria tenuis* is host nonspecific while AK-Toxin and AM-Toxin produced by *A. Kikuchiana* and *A. mali* are host specific (Agrioe, 1988). The toxin is effective even at a concentration of 2 ppm. It is a cyclopeptide with molecular formula of $C_{24} H_{32} N_4 O_4$ (Singh, 1987). Peso and Main (1970) isolated alternariol monomethylether from *A. tenuis* while Meronuck *et al.*, (1972) recorded tenuazonic acid, a toxin produced by *Alternaria alternata*.

The production and effect of tentoxin has also been studied by Fulton *et al.*, (1965), Templeton *et al.*, (1967) and Siddaramai *et al.*, (1984).

Narain and Das (1976) adopted a bio-assay method based on the inhibition of root elongation, whereas toxicity was determined in terms of percentage inhibition of seed germination, fruit spoilage by Raja and Pillayarswamy (1972). Kumar and Mehmood (1986). Progressive inhibition of seed germination root elongation and production of host non-specific toxin have also been observed by Meehan and Murphy (1947) and Sahni *et al.*, (1974).

10. Changes in nutritive value during patho-genesis

Fungal infection on the host tissue results in great shifts in the nutritive contents. Changes in ascorbic acid during pathogenesis of four tomato fruit rot fungi, *Phoma exigua*, *Rhizoctonia solani*, *Stemphylium vesicarium* and *Niorospora oryzae* were studied and drastic reduction of ascorbic acid in diseased fruits was noticed by Reddy *et al.*, (1980).

Rapid decline in ascorbic acid during the pathogenesis of chilli fruits due to *Choanephora cucurbitanum* was noticed by Chahal and Grover (1972). Reduction in amino acid content was also recorded by Shivaprakasam *et al.*, (1972).

Decrease in capsaicin content and ascorbic acid has been reported by Awasthi and Singh (1975) in chilli fruits infected with cucumber mosaic vines.

Capsaicin content of chilli fruits has been determined by several workers. Wide variation in the shape, size and age of fruits has been related with capsaicin content. It is present in placental tissue and pericarp (Deb *et al.*, 1963; Ramanujam and Thirmalachar, 1966;

Thirumalachar, 1967; Charbawi, 1977; Bajaj *et al.*, 1978; Azad, 1991; Tiwari, 1990). *Alternaria* infection brings the changes in the nutritive value of the crop. It has been observed that in association with other fungi, *Alternaria alternata* decreased the starch (carbohydrate) content of pigeonpea seeds (Sinha *et al.*, 1981).

11. Management of the disease

Chilli crop has been observed to suffer due to preand post-emergence rots, leaf spots, fruit rot and tender tip drying incited by *Alternaria alternata* (Verma and Bhale, 1989). Havy losses are incurred in the nursery stage and furit stage. Attempts have been made by several workers to find suitable measures for the management of the disease (Courter *et al.*, 1965; Takeno *et al.*, 1985; Chauhan and Duhan, 1986; Dhyani *et al.*, 1990).

Evaluation of germplasm and chemical control methods are the general and well adopted approaches, however, recently in Tamil Nadu, Sujatha Bai *et al.*, (1993b) have attempted some non-chemical methods. Fungitoxicity of extracts of 10 botanical families and plant products was recorded under pot conditions. Two spray of the leaf extract (10 per cent) of vilvum (*Aegle maremblos*) (Bail) combined with 0.01 M. nickel sulphate at 100 and 115 days after sowing significantly reduced the disease intensity.

Takano *et al.*, (1985) verified the influence of dry heat for control of seed-borne diseases, although complete control of the associated fungi was achieved but impaired germination of seeds is the main rejection point of this approach, Mathur and Agnihotri (1961) advocated the simple method for reducing the losses in chilli caused by *Alternaria alternata*.

They suggested to store the fruits at 16-26°C and cautioned to take care during picking.

11.1 Evaluation of chilli cultivars

Search for management of the disease through selection and breeding of different genotypes has also been a major area of research in the management strategies. Few attempts have been made to evaluate the cultivars under artificial inoculation or natural field conditions exclusively against *Alternaria* fruit rot (Singh, 1987). Some attempts have been made for other diseases of chillies (Bansal and Grover, 1969; Kadu *et al.*, 1978; Ullesha *et al.*, 1981).

At Hissar, Chauhan and Duhan (1986) evaluated 87 lines/varieties of *Capsicum* against anthracnose, *Alternaria* blight, *cercospora* leaf spot and leaf curl. In former USSR, at Noskova, Melikova (1960) had made a search and evaluated several entries and succeeded to find out relatively resistant varieties. Similarly in USA, Courter *et al.*, (1965) studied the field susceptibility of pepper varieties and made selections to fruit rot caused by *Alternaria tenuis*.

In Tamil Nadu, two highly resistant chilli genotypes CA 87-4 and CA 74-8 were identified against fruit rot caused by *Alternaria tenuis* (Sujatha Bai *et al.*, 1993a). Singh (1987) observed that Bunchi cv. Was least susceptible among 16 cultivars tested against the disease.

11.2 Seed-treatment

The use of chemicals for seed dressing was recommended by several workers for the reduction of load of seed mycoflora, including *Alternaria* sp. (Neergaard, 1977; Suryanarayan, 1978).

Jharia *et al.*, (1977) conclusively established the effectiveness of seed dressing with Thiram + Captan (1:1) 0.3 per cent by weight in checking the pre and post emergence losses and mortality at adult stage in

chilli under Banda conditions. Higher yields were recorded due to the treatment.

The efficacy of Ferbam, Maneb and Captan at 0.5 to 0.25 per cent was confirmed by Crisan and Mesescu (1970) while investigating the leaf chlorosis caused by *A. alternata* on red pepper.

In Romania during 1976, extensive rotting of red pepper caused by *Alternaria alternata* was successfully controlled by three sprays of Thiram (0.3%) resulting in only 4.3% infection as compared to 51.5% in untreated plants (Tutunaru and Raicu, 1978).

In India, Mali and Joi (1985) found Thiram, Captafol and Vitavax most effective of chilli including *Alternaria alternata* whereas Dhyani *et al.*, (1990) used seven fungicides for controlling 26 fungi associated with chilli seeds. They observed Bavistin, Captafol, Thiram, Dithane M-45, Topsin-M and Vitavax the best.

Chemical seed treatment studies for controlling associated *Alternaria spp.* have been conducted. Efficacy of Thiram in reducing the association of *Alternaria alternata* was recorded by Ellis *et al.*, (1977), Ellis and Paschal (1979) while Kannaaiyan *et al.* (1980) recorded the best results with Senlate-T at 3g/Kg seed in pigeonpea. In Bangladesh, Mridha and Chowdhury (1990) observed the efficacy of Benomyl, Mancorab and Vitavax 200 against the seed borne natural infection of *A. alternata* and *C. capsici* at 0.45 per cent after six days.

Siddaramaiah *et al.*, (1980) recommended the use of Thiram or Captan (0.3%), while Prasad (1985) observed Triforine (Saprol) 0.15% the best out of ten fungicides Carbendazim (Bavistin), Tridimenol (Bayton), Carboxin (Vitavax), MBC, Captan, Cerasen, Chlorothalonil (Deconil), Manoozeb (Dithane M-45) and Thiram as seed treatment against *Alternaria carthami* in safflower. In Pakistan, Sultana *et al.*,

(1988) found Captan and Benomyl were most effective against *A. alternata*.

11.3 Foliar spray treatment

Alternaria alternata has been reported to cause leaf spots, blight and fruit rot. The disease has been observed in red, semi-red and green fruits of chillies. Combination of seed treatment and subsequent spray of chemicals has been found quite effective.

Jharia *et al.*, (1977) recorded four spraying of zineb (0.25%) very effective against foliar diseases including *Alternaria sp.* infection, out of eight fungicides tested. Higher yields were also obtained after treatment. Seed treatment with Thiram + Captan (1:1) 0.3% and four sprays of zineb (0.25%) were found to be the best. Early blight caused by *Alternaria* at nursery stage was effectively controlled by the application of yellow copper oxide in Mexico (Brauer and Richardson, 1957).

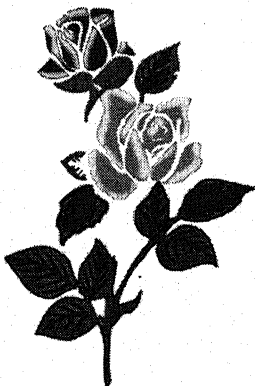
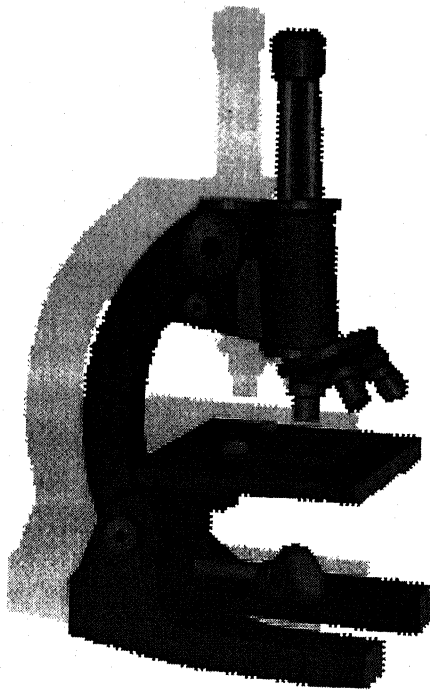
In west Pakistan, reduction in fruit rot infection was reported by the application of Sandoz-Copper and Dithan. The effectiveness of copper sulphate preparations as Bordeaux nixture was demonstrated (Perwaiz & Kirti *et al.*, 1968). Copper was effective under laboratory conditions against *Altemaria Alternata* (Singh and Tandon, 1967).

The fungus also poses a problem during post harvest handling. It has been found associated with sun-dried chillies as internal moulds (Leyendecker, 1954b). Zerlate and Dithane were found promising in New Mexico.

In USA, inhibition of *Alternaria* rot of bell pepper (*Cepricum annums L.*) was recorded by dipping the fruits for 10 minutes in water solution of 50-250mg a.i. CGA64251 or Inaxalli (Spalding and King, 1981). Fruit treatment with chiorine and Imazalil dip was also found better (Miller *et al.*, 1984).

Seed bed treatment with Ziram or Captan and spraying of chilli crop when fruits are about half mature with Zineb at 7-10 days interval controlled the *Alternaria* fruit rot effectively (Shurtleff & Linn, 1963).

MATERIALS & METHODS



MATERIALS AND METHODS

The Present investigation was undertaken to study the epidemiology and management of leaf spot and fruit rot of chillies incited by *Alternaria alternata*. The materials used and methods followed are described below.

1. General

1.1 Cleaning and sterilization of apparatus

The glasswares used during the course of investigation are of Corning and Borosil make. Prior to use, each glass are was cleaned with chromic acid solution (sulphuric acid 300ml, potassium dichromate 80g and distilled water 400ml) followed by thorough washing with detergent powder and finally rinsed with normal tap water as per need. The dried glasswares were sterilized in an electric hot air oven at 180°C for 120 minutes. The media ware sterilized in an autoclave at 1.05 kg cm² (15 lb per square inch) for 15 minutes, whereas soil at 1.05kg cm for two and a half hours. Plastic pots were sterilized with 0.1% Mercuric chloride solution, followed by thorough washing with sterilized water. Surface sterilization of chilli plant parts and chilli seeds was done by dipping them in 0.1% mercuric chloride solution for 60 to 90 seconds, followed by three regular changes of sterile distilled water. Sterilization of earthen pots was done by dipping in 5% copper sulphate solution, followed by thorough washing with sterile water.

The inoculation needles, forceps and biological needles were sterilized by dipping them in alcohol and heating over a flame.

The Petridishes containing the medium were stored for 24 hours inside the inoculation chamber prior to use to avoid the possibility of

contaminations. Inoculated plates were exposed to 40w Philips day light tubes for 6 hours every alternate days. Virulence of the isolated *Alternaria alternata* was tested every alternate month by inoculating healthy chilli fruits by pin prick method and incubated at 25°C.

1.2. Media

The ingredients of the media used during the course of investigation are given below.

1.2.1. Non-synthetic media

(i) Potato dextrose agar medium (PDA)

Peeled and sliced potato	200g
Dextrose (anhydrous)	20g
Agar-agar	20g
Distilled water	1000ml

(ii) Rice meal agar (RMA)

Rice meal	20g
Agar-agar	20g
Distilled water	1000ml

(iii) Corn meal agar (CMA)

Corn meal agar	20g
Distilled water	1000ml

(iv) Soil extract agar

Soil extract agar	100ml
Glucose	1g
Dipotassium phosphate	0.5g
Agar-agar	20g
Sterile water	900ml

Soil extract was prepared by steaming 1000g of garden soil in tap water in an autoclave at 1.05kg/cm² for two and a half hours. A Small

quantity of calcium carbonate was added to it and soil suspension was filtered through a whatman 42 double filter paper. The turbid filtrate was then poured into a flask and sterilized and made 900 ml by adding sterile water.

(v) Chilli seed extract agar (CSE)

Chilli seed extract	200g
Glucose	20g
Agar agar	20g
Distilled water	1000ml

Two hundred gram chilli seed was ground and boiled, then the extract was passed through muslin cloth.

(vi) Oat meal agar (OMA)

Fifty gram oat meal was boiled in 300ml water till quantity of water remained half and volume was made upto 1000ml and 20g agar-agar was added.

(vii) Soil-maize medim (SMM)

Soil + Sand (1:1)	95g
Ground maize	5g
Distilled water	30ml

1.2.2. Synthetic media

(viii) Asthana and Hawker's medium

Dextrose	5g
Potassium nitrate	3.5g
Potassium dihydrogen phosphate	1.75g
Magnesium sulphate	0.75g
Agar-agar	20g
Distilled water	1000ml

(ix) Czapek's agar

Potassium dihydrogen phosphate	1.75g
Sucrose	20g
Sodium nitrate	2g
Magnesium sulphate	0.5g
Potassium chloride	0.5g
Ferrous sulphate	0.01g
Agar-agar	20g
Distilled water	1000ml

(x) Richard's agar medium

Potassium nitrate	10g
Potassium dihydrogen orthophosphate	5g
Magnesium sulphate	2.5g

(xi) Martin's medium

Dextrose	10g
Peptone	5g
Potassium dihydrogen phosphate	1g
Magnesium sulphate	0.5g
Agar-agar	20g
Distilled water	1000ml

1.3. Incubation chamber

To provide optimum conditions for the germination of seeds and growth of mycoflora associated with chilli seeds, petriplates containing three moisture blotters and 25 seeds plated on them were incubated in a wooden growth chamber during the course of study. The dimensions of the chamber were length 3m, width 0.75m and height 0.9m. Two sets of Philips 40w day light tubes were provided in the chamber horizontally at a height of 40 cm. Alternate cycles of 12 hr light and 12 hr dark periods

were maintained. One of the sides of the chamber was provided with a water cooler to maintain the temperature at 28⁰C and to minimise the losses of water from blotters and medium in the petridishes.

1.4 Meteorological data

Data on rainfall, relative humidity, maximum and minimum temperature were recorded from the meteorological observatory at Department of Meteorology IGFR, Jhansi.

1.5 Field Experiments

Observations on the field experiments were recorded from IGFR, Jhansi till 2005.

METHODS

1. Survey

To study the distribution and occurrence of chilli at regular interval survey were conducted at 20 locations around Banda during 2002-03 and 16 locations of Jhansi during 2003 and 2004. One square meter (1x1m) was thrown randomly at 10 points in a field and total infected plants within the square were counted to calculate the disease incidence. The average of 10 observations was calculated as below:

$$DI = (TIP \times 100) / TPO$$

Where,

DI = Disease incidence i.e. per cent plant exhibiting symptoms.

TIP = Total number of infected plants.

TPO = Total number of plants observed

Infected plants and diseased plant parts were collected in envelopes and brought to the laboratory and observations were recorded on the locality, variety, crop stage, soil type, crop pattern and disease incidence.

2. Collection of chilli seed samples and diseased parts

During survey, 20 seed samples from each place were collected representing the specific locality. Seed samples of 19 varieties/lines were also obtained from the Scientist (Horticulture), Deptt. of Horticulture, C.S.A. University of Agri. & Tech., Kanpur. Eleven seed samples were collected from I.V.R.I., Varanasi. Overall 50 seed samples of chilli were selected and categorized into two groups:

- (i) Seed samples variety-wise.
- (ii) Seed samples locality wise.

Seed samples were numbered and stored in paper envelopes at 4°C to avoid deterioration.

3. Symptomatology

Symptoms produced by *Alternaria alternata* on chilli seeds, seedlings, leaves and fruits were studied.

3.1. Seeds

Chilli fruits (Jawhar Mirch 218 and Pusa Jwala) naturally infected with *Alternaria alternata* were collected from fields and graded into no infection, slight infection (1-10%), moderate infection (10-25%) and severe infection (26% and above). The fruits were kept in refrigerator (4°C) for two months. Seeds were collected separately and were used for study. Seeds were stored in wax paper envelopes at room temperature.

3.2. Seedlings

Symptoms produced on chilli seedlings were studied under artificial conditions by raising seedlings in soil infested with *Alternaria alternata*, while proving pathogenicity and under natural conditions where seedlings were grown on seed bed.

3.2.1. *In Vitro*

Soil maize medium was prepared 100g in each 250 ml conical flask infested with *Alternaria alternata* and incubated at 25⁰C for 30 days. Culture of one flask was mixed with 1Kg sterile soil. Aluminium trays (51 x 34 x 1cm) were filled with such infested soil. Suitable control i.e. trays with sterile soil (uninfected) were maintained. Surface sterilized chilli seeds of Pusa Jwala were sown in the trays. One hundred seeds were sown in each row. The trays were irrigated every day after sowing (150ml/tray). After 10 days of sowing, the seedlings were covered with perforated polythene bags for 3 days. The symptoms on the trays were examined upto 30 days.

3.2.2. *In vivo*

Seed beds identified to be naturally infested during survey were used for the study. Surface sterilized seeds were sown and symptoms were observed at different growth stages and isolations were made from the plant parts.

3.3. Leaves

The leaf spot disease frequently occurred on chillies every year in the vicinity of Banda and Janshi region. Diseased leaves from different localities/varieties were collected and studied for the association of the fungus.

3.4 Fruits

Naturally infected fruits of different varieties and stages were collected and symptoms incited by *Alternaria alternata* were studied and isolations were made.

4. Isolation, purification, identification and maintenance of *Alternaria*.

On fruits and leaves exhibiting typical symptoms of the disease were collected from different locations and brought to the laboratory for isolation. The diseased plant parts were cut into small pieces and surface sterilization with 0.1 per cent mercuric chloride solution for 30 seconds, followed by three washings and then small disease parts put on 20 ml solidified PDA. The Petridishes were incubated at $25 \pm 1^{\circ}\text{C}$. Isolations were made from surface sterilized plant parts. The fungus was subcultured, purified with fungal hyphal tip method and single spore method (Exekiel, 1930). The identification of the isolated fungus was done on the basis of morphological characters (Subramanian, 1971; Ellis, 1971).

5. Pathogenicity Test

Pathogenicity of the isolated *Alternaria alternata* was tested by three ways; (i) seed infestation, (ii) soil infestation, and (iii) fruit inoculation.

5.1. Standardization of time required for maximum conidial production

The standardization of duration required for maximum conidial production of *Alternaria alternata* was done prior to pathogenicity test. The fungus was grown on PDA in petridishes for 6, 8, 10, 12, 14, 16 and 18 days at 25°C . The Petridishes were inoculated with a 5mm culture disc. After completion of incubation periods, the observations on conidial production were recorded.

A 5mm culture disc was transferred to the test tube containing 10 ml of sterile water. The suspension was prepared by shaking the tube gently. The number of conidia per ml of suspension was determined with the

help of a haemocytometer. The incubation period required for the maximum conidial production was followed for multiplication of the inoculum.

5.2. Seed infestation technique

5.2.1. Eight day old culture of *Alternaria alternata* grown on potato dextrose agar medium was used. Seed samples free from natural infection of *A. alternata* were used in the test. The seeds were pretreated with 0.1% mercuric chloride solution for 60 seconds, washed thoroughly with sterile water and soaked in water for 120 minutes. Soaked seeds were rolled in actively grown culture of the test fungus. Ten such infested seeds were placed on moist blotters in petridishes. One hundred seeds were used. Seed without fungal infestation served as control. Petridishes containing these seeds were incubated in the chamber and were observed for the development of disease symptoms after 7 days.

5.2.2. Seed infestation method was tried in another way, where the seed coat of pretreated chilli seeds was injured with the help of sterilized needle and then rolled on the actively grown culture of *A. alternata*. Seeds were placed on moist blotters as in 5.2.1. Seeds injured but without infestation were kept simultaneously as control. Observations on the disease development were made after seven days of incubation.

5.3. Soil-infestation method

Inoculum of *Alternaria alternata* was grown in 250 ml Erlenmeyer conical flasks each containing 100ml potato sucrose broth. After sterilization of the medium, 5mm disc of seven day old culture was transferred in each flask and incubated at room temperature $25 \pm 1^{\circ}\text{C}$ for 15 days. After incubation, mycelial mat was removed and washed thoroughly in sterile water to remove the traces of culture filtrate and mixed in sterile soil at the rate of the fungus of one flask per 200g. The

infested soil was thoroughly mixed and was filled in plastic pots (10 cm diameter). The fungus was allowed to grow for 10 days at room temperature. Ten surface sterilized seeds with no natural infection of *Alternaria alternata* were sown in each pot. Un-infested sterilized soil served as control. Observations were recorded on the development of the disease in seeds and seedlings. Isolations were made from the infected seeds and seedlings on PDA medium for the associated fungi.

5.4. Fruit and leaf inoculation method

Four methods were used to test the pathogenicity of the fungus on fruits (ripe and turning red stage) and leaves under laboratory and field conditions on intact and detached parts. Spore suspension was prepared by adding 10ml sterile water in 8 day old culture grown in test tube. The inoculated leaves and fruits were covered with belljar and humidity was maintained with the help of moist cotton placed inside the belljar. In field, inoculated fruits and leaves were covered with polythene bags with moist cotton.

5.4.1. Pin prick method

Ten pin heads (entomological needles) were mounted on cork covering 1 sq. cm area and five pricks were given on each intact and detached leaf and fruits prior to spray of spore suspension of the fungus.

5.4.2. Spore suspension spray method

Spore suspension of *Alternaria alternata* was sprayed with the help of atomizer on dorsal and ventral side of chilli leaves and in case of fruits entire surface was covered by spraying the spore suspension.

5.4.3. Carborandum rub method

Spore suspension spray method was modified by making injury with the help of carborandum and the spore suspension was sprayed with the help of an atomizer on detached and intact leaves and fruits.

5.4.4. Tooth prick method

Tooth prick with small bits of mycelial mat was inserted in the fruits and observations were recorded on the symptom development.

The inoculated leaves and fruits were kept in sterile clean petridishes under laboratory conditions and provided with 12hr dark and 12 hr light periods at $25 \pm 1^{\circ}\text{C}$. Humidity was maintained with the help of a wet-cotton piece placed with petridishes containing inoculated leaves/fruits under belljars. Under field conditions, temperature ranged from $20\text{-}30^{\circ}\text{C}$ while humidity was 40-60%.

6. Detection of *Alternaria alternata* and other mycoflora associated with chilli seeds:

6.1. Standard Blotter Method (ISTA, 1985)

All the collected seed samples of chilli from different localities and varieties were tested by Standard Blotter Method (ISTA, 1985) for the mycoflora associated with chilli seeds. Three circular pieces of blotter papers of the size of petridish were cut and dipped in sterilized water, excess water was drained off and placed in each sterilized petridish. Twenty-five chilli seeds were placed in each petridish with the help of sterile forceps under sterile conditions in the inoculation chamber (16 seeds in outer circle, eight in the inner and one in the centre) so as to allow equal distance between seeds. The plated petridishes were kept for incubation in the growth chamber. Two hundred petridishes treated and untreated with 0.1% mercuric chloride for 30 seconds and then washed in three changes of sterile water. Petridishes were examined on the eighth day of incubation. Fungi were observed and identified by making slides and on the basis of colony and habit characters under stereoscopic binocular.

6.2. Naked eye examination

Seed samples of four selected seed samples were examined by naked eye and were classified into following groups :

- A = Bold apparently healthy seeds
- B = Deformed shrunk and brown seeds

The classified seeds were incubated on moist blotter as in Standard Blotter Method (ISTA, 1985) without any pretreatment.

7. Efficacy of various methods in the detection of *Alternaria alternata* and other important fungi associated with chilli seeds:

The following methods were tested for the association of *Alternaria alternata* with four selected seed samples of chilli obtained from Jhansi, Hamirpur, Mahoba and Banda exhibiting the maximum natural infection of the fungus. Two hundred seeds of each sample were tested by each of the following methods:

- (i) Standard Blotter Method (ISTA, 1985)
- (ii) Standard Agar-plate Method (Modified Ulster method Muskett and Colhoun, 1948)
- (iii) 2, 4-D Method (Neergaard, 1973)
- (iv) Deep Freezing Blotter Method (Limonard, 1968)

7.1. Standard Blotter Method

Standard Blotter Method (ISTA, 1985) was used for the detection of mycoflora associated with four selected seed samples of chillies. Surface sterilized and unsterilized seeds were used. On the eight day of incubation the seeds were examined for the association of fungal flora. The details of the method is given in 6.1.

7.2. Standard Agar plate Method

In this method, potato dextrose agar medium was used, instead of previously used malt extract. In each sterilized petridish, ten pretreated seeds were placed with the help of sterilised forceps on 20 ml solidified PDA medium at equal distance, nine seeds in outer ring and one seed in the centre. Observations on the associated fungi were recorded on the fifth day of incubation with the help of making slides of growing fungi.

7.3. 2, 4-D Method

7.3.1. Chilli seed soaking method

Sterilized petridishes were prepared as in Standard Blotter Method. The seeds were dipped in 2000 ppm of solution of 2, 4-D (Sodium salt of 2, 4-dichlorophenoxy acetic acid) in muslin cloth for 15 minutes, then 25 seeds were plated on moist blotter in each petridish and incubated for seven days in the incubation chamber. Observations were recorded on the associated mycoflora.

7.3.2. Blotter dip method

Three circular pieces of blotter, as used in Standard Blotter Method, were dipped in 2000 ppm solution of 2, 4-D and after dripping off the extra solution, these were placed in each sterile petridish. Twenty-five seeds were plated on the moist blotters. The seeds were examined for the presence of fungi associated after incubation period of seven days. In this method, sterile water was replaced by 2, 4-D solution to dip the blotters.

7.4. Deep freezing blotter method

The procedure for preparing petridishes (with moist Blotters and 25 seeds) was similar to the Standard Blotter Method, After 24 hr of incubation, the petridish containing seeds were transferred to the deep freezer at -7°C in complete darkness. Petridishes were taken out after 24

hr an kept again in the chamber for incubation. On the seventh day of incubation, the seeds were examined for the associated fungi.

8. Use of modified methods for the detection of *Alternaria alternata* associated with chilli seeds

8.1. Modification of Standard Blotter Method

Modification of Standard Blotter Method was tried by changing the initial hydrogen ion concentration (pH) of water used for wetting the blotters. Three levels of pH i.e. 6, 7 and 8 were adjusted with the help of 0.1 N NaOH and 0.1 NHCl.

8.2. Modification of Standard agar plate Method

Modification of agar plate method was tried by incorporating chilli seed extract in potato sucrose agar medium. Rest of the method was the same to that of agar plate method. Observations on the fungi associated with chilli seeds were recorded on fifth day of incubation.

8.3. Modification of Deep-freeze blotter method

Modification of deep freeze blotter method was tried in which the blotters were soaked in solution of antibiotics instead of plain sterile water. The blotters were dipped in 200 ppm solution of strepto mycin. Water without addition of antibiotics served as control. The seeds exhibiting the presence of *A. alternata* were recorded after seventh day of incubation.

9. Factors influencing growth and sporulation of *Alternaria alternata*

9.1. Effect on growth

9.1.1. Effect of media

In all ten media were used to find the influence on the growth and sporulation of *A. alternata* isolated from infected chilli fruits. The media used were Potato-dextrose agar (PDA), Rice-meal-agar (RMA), Corn-meal-agar (CMA), Soil-extract-agar (SEA), Chilli seed extract agar (CSE), Oatmeal-agar (OMA), Czapek's agar (CA), Asthana and Hawker's agar (AHA), Richard's agar (RA) and Martin's agar (MA).

Media were prepared and sterilized in an autoclave and 20ml lukewarm medium was poured into each sterilized petridish. 7mm discs from 8 day old culture of *A. alternata* were cut, one disc was placed in the centre of each petridish and incubated at 25°C. Colony growth was measured and cultural characters were recorded after 5th and 8th day of incubation. For determination of spore production, ten mm disc was cut with the help of cork borer and suspended in 5ml of water in a test tube. the disc was macerated and the suspension was used for counting the spores per microscopic field. Further desired calculations were made.

The procedure of testing was the same as the Standard Blotter method (ISTA, 1985). Observations on the association of *Alternaria alternata* were recorded on the eighth day of incubation.

9.1.2. Effect of temperature

Petridishes containing PDA and inoculated with seven mm culture disc of *A. alternata* were incubated at 20, 25, 30 and 35°C. Observations were recorded on the colony character and sporulation.

9.1.3. Effect of hydrogen ion concentrations (pH)

Eight pH levels (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) were used to study the influence of pH on growth and sporulation of *A. alternata* on PDA. Seven mm disc was cut from 8 day old culture and placed in the centre of petridish. Petridishes were incubated at 25°C. Colony characters

were studied. Conidial pH was adjusted with the help of 0.1 N HCl and 0.1 N NaOH before sterilization.

9.2. Effect on spore germination

Cavity slide method was employed for studying the spore germination of *A. alternata* isolated from chilli fruits of variety Pusa Jwala. Conidial suspension was made in sterile water in culture tube and standardized to about 15 conidia under low power of compound microscope. One ml of the conidial suspension was placed in each of the three cavities of each slide. Four slides were kept for each treatment.

The slides were kept in moist chamber prepared by using petridishes provided with moist blotter and glass rods. The data on germination of spores were recorded.

9.2.1. Effect of temperature

With a view to see the effect of temperature on spore germination, the cavity slides with spores were kept in the Petridishes at 15, 22, 25, 27 and 30°C. The data on spore germination were recorded.

9.2.2. Effect of relative humidity

Four humidity levels (90, 75, 50 and 25) were used in the experiment. The humidity levels were maintained by using saturated solutions kept in the desiccators.

90%	Distilled water
70%	NaCl + KCl equivalent parts
50%	(NH ₄ NO ₃ + KNO ₃ equivalent parts)
40%	KNO ₂

The cavity slides were kept in the sterile desiccators in which the different humidity levels were maintained. The spores were kept dry and placed at different relative humidities.

9.2.3. Effect of exogenous supply of suger

In order to find the effect of exogenous supply of carbohydrate, various concentrations of sucrose ranging 0.01, 0.25, 0.5, 1.0 and 1.5% were used. Distilled water, without sucrose served as control. The observations were recorded on spore germination upto 5 hours.

9.2.4. Effect of seed-exudates

Five gram of seeds of chilli of Pusa Jwala were soaked in 20 ml distilled water in 250ml Erlenmeyer conical flask and kept for germination at room temperature. After 5 days, the water was decanted and passed through sterile unit of Seitz filter. The filtrate was tested for its effect on germination of spores of *A. alternata*. The spore suspension was prepared in the filtrate. One drop of the suspension was placed on a cavity slide with the help of sterile pippette. After 5 hours, the final germination was recorded.

10. Transmission of *Alternaria alternata*

Transmission of the fungus on the host from one season to another season was studied.

10.1. Seed to plant

10.1.1. Role of seed-borne infection in causing diseases

Role of *A. alternata* in causing seed and seedling diseases was determined by the method recommended by Khare *et al.* (1977). In this method, 10ml hot plain water agar of one per cent strength was poured in each of the 100 *rimless* test tubes of 30ml capacity. Mouth of test tubes was closed with slightly loose cotton plugs and autoclaved. After solidification of the medium, one seed of sample No. 27 was placed in each tube with the help of sterile forcep on water agar. The tubes were plugged again and kept in growth chamber for incubation. The plugs of

the tubes were removed when seedlings reached the top. Observations were recorded on the seed infection and seedling infection due to *A. alternata* after 12 days.

10.1.2. Effect of seed-borne *A. alternata* was also determined by sowing the seeds in sterilized sand filled in sterilized plastic pots of 15 cm diameter. Observations were recorded on the infection on seed and seedlings. In each pot, 10 surface sterilized seeds were sown and observations were recorded after 20 days. The pots were provided alternate cycles of dark and light periods of 12hr. The infected seeds and seedlings were examined under microscope and isolations were made on PSA medium for the association of *A. alternata*.

10.1.3. Location of *A. alternata* inside the chilli seeds (site of infection)

10.1.3.1. Seed component plating

The method recommended by Sinha and Khare (1977) was adopted to locate the site of infection.

10.1.3.2. Location of *A. alternata* was confirmed by histopathological studies after cutting the graded section of leaf and stem of chilli plant. Following steps were adopted for histopathological studies.

10.1.3.3. Fixation of samples

Samples infected due to the fungus were collected, cut into 5x5 and 5x2 mm with the help of a sharp knife and fixed in FAA solution (Formalin 40%-5ml, Acetic acid glacial-5ml and Ethyl alcohol 50%-90ml).

10.1.3.4. Dehydration

Fixed samples were washed thoroughly to remove the traces of FAA and then passed through the following series for one hour in each solution :

25% alcohol 50% alcohol 70% alcohol

90% alcohol absolute alcohol

10.1.3.5. Infiltration

Samples kept in absolute alcohol were passed through alcohol: xylene series for 1 hour in each solution.

25% xylene, 50% xylene, 70% xylene, pure xylene

10.1.3.6. Embedding

Parafin wax 58-60°C (E. Merck, Bombay) was used for making blocks.

10.1.3.7. Sectioning

Prepared wax blocks containing infected samples were mounted on rider and sectioning was done by rotatory microtome (Spencer, USA). Transverse sections were cut of 15-25µ thickness. Clean microslides were coated with Haupt's adhesive and ribbons were placed on it. Warming of these slides was done after flooding them with 4% Formalin solution for 3 minutes.

10.1.3.8. Staining

One set of slides were processed for mounting directly and another set of slides were passed through xylene Alcohol series and then alcohol: distilled water series (backward series) for 2 minutes in each series. Sections were stained in 0.5% safranin aqueous solution for 5 minutes. Stained sections were again passed through forward series (2 minutes in each) upto xylene series.

10.1.3.9. Mounting

Slides taken out from xylene pure were immediately mounted with 2 or 3 drops of Canada Balsam. Rectangular cover slip was placed before taking microscopic observations, the slides were kept in over (60°C) for overnight.

10.2. Plant to seed

10.2.1. Fruit Inoculation Technique

Fruits of different age of Pusa Jwala were inoculated with 3ml of spore suspension of *A. alternata* of 8 day old culture through hypodermic syringe (Needle No. 22) and covered with polythene bags containing wet cotton to provide moisture/humidity for 6 days, under field conditions. Sterile water was injected in fruits served as control. At maturity, seeds of such fruits were collected and plated on PDA medium after surface sterilization. Similarly, seeds of naturally infected fruits were collected and tested for association of *A. alternata* by blotter method as well as PDA method.

10.2.2. Intected plant parts like twig, stem, fruits and leaves were collected and observed for the association of *A. alternata* under microscope and isolations were made.

10.2.3. Naturally infected stem, twig and fruits were collected, initially checked under microscope for the association of the fungus and stored in paper envelops under room temperature for periodical observations and isolations were made for the associated fungus.

10.2.4. Naturally linfected twigs, stem and fruits were mixed and cut into small portions and burried in the field soil placed in the pots. Isolations from the small bits of those burried plant debris were made on PDA. Observations were recorded on the survival and viability of the fungus.

11. Influence of toxin produced by *Alternaria alternata* on the seed germination and root-shoot length

11.1. Preparation of culture filtrate

A bio-assay method based on the inhibition of root shoot elongation was planned (Das and Shrivastava, 1969). Toxicity was determined in terms of inhibition of seed germination and root/shoot elongation on chilli seedlings over check. Richard's medium was used as the basal medium and pretreated healthy chilli seeds having no natural infection of *A. alternata* were used. In each 150ml Erlenmeyer flask, 50ml of the medium was taken and autoclaved. Five mm disc of culture was placed in each flask. Flasks were prepared for six incubation periods 5, 10, 15, 20, 25 and 30 days. Flasks were incubated at room temperature $28 \pm 1^{\circ}\text{C}$. After the incubation period the medium was filtered through muslin cloth, cotton pad followed by Seitz filter, to obtain a bacteria free culture filtrate. The crude culture filtrate (CF) was considered as 100%. Twenty-five pretreated seeds were placed in sterile petridishes containing 5, 10, 15, 20, 25 and 30 day old culture filtrate. The culture filtrate exhibiting maximum inhibition of chilli seed germination was further tested at different dilutions. Seeds were soaked in culture filtrate for 3 hours and plated on blotter. Seed soaked in sterile water for the same period served as control.

The culture filtrate dilutions were prepared with sterile water. The observations on seed germination, root shoot length were recorded after 72hr. Thermal inactivation period was also determined.

11.2. Dilutions of culture filtrate

Various dilutions viz. 1:1, 1:2, 1:3, 1:4 and 1:5 of the culture filtrate of 15 day old were made with sterile distilled water. Three blotters were soaked and placed in each petridish. Seeds of Pusa Jwala were used after surface sterilization with mercuric chloride (1:1000) solution, followed by three washings of distilled sterile water to remove the traces

of mercuric chloride. Twenty-five seeds were plated in petridish as in Stabdar Blotter Method (ISTA. 1985). Germination was recorded after 13 days of incubation. Blotters soaked in sterile Richard's medium corresponding dilutions with sterile water served as control following the method adopted by *Meehan and Murphy* (1947).

11.3. Determination of absorption time of toxic metabolite by chilli seeds

In order to know the time taken for absorption of toxic metabolite by seeds, the pretreated seeds of Pusa Jwala were soaked for 1, 2, 3, 6, 9 and 12 hr in culture filtrate of *A. alternata*. Immediately after soaking, the seeds were washed with distilled water three times so as to remove the excess of filtrate present on the surface of seeds. Twentyfive treated seeds were placed in each petridish containing three moist blotters. The petridishes were incubated for 7 days in growth chamber. Seeds soaked in Richard's medium for 1, 2, 3, 6, 9 and 12 hours as their respective control. Inhibition in germination indicated the presence of toxic metabolite in the seeds.

11.4. Influence of temperatures on the activity of toxic metabolite

Culture of *A. alternata* was grown on Richard's medium for 15 days at room temperature. Culture filtrate was sterilized by filtering it through Sietz filter, and diluted with distilled water (1:1). Fifty ml of this culture filtrate was transferred to 250ml Erlenmeyer flask. These flasks were placed in water bath at 30, 40, 50, 60, 70, 80 and 90°C for 10 minutes. After cooling, the volume of each flask was made upto 50ml (where required) with distilled water. Three blotters were soaked with such solution and placed in petridishes. Twenty-five seeds were placed in each petridish and incubated for 7 days in growth chamber. Reduction of

inhibition in chilli seed germination indicated the degradation of toxic metabolite. Four replications were kept.

12. Factors affecting disease development

12.1. Under field conditions

Observations were recorded on the development of fruit rot and twig infection and relation between temperature and humidity was worked out at Janshi and Banda conditions.

12.2. Under laboratory conditions

12.2.1. Influence of temperature and relative humidity on the disease development was determined under laboratory conditions. Fruits of Pusa Jwala and Jawahar Mirch 218 were brought to the laboratory and inoculated with pin prick method. The inoculated fruits were exposed to 10, 15, 20, 25, 30 and 35°C and 50, 60, 70, 80, 90 and 100% relative humidity levels. Observations were recorded on the development of fruit rot at 10 and 15 days after inoculation. In each treatment 100 fruits were inoculated.

12.2.2. Influence of stage and various age of fruits on infection was determined by collecting the fruits of Pusa Jwala and Jawahar Mirch 218. Inoculation was done by pin prick method. The fruits were kept at 25°C. Observations were recorded after 15 days. Fruits of eight stages were collected. Green, green yellow (dominating green) I, green yellow (yellow dominating) II, complete yellow, yellow red (yellow dominating) I, yellow red (red dominating) II, complete red and full ripe red fruits of almost equal size and shaps were inoculated.

13. Estimation of capsaicin content in chilli fruits

Capsaicin content of naturally infected chilli fruits and healthy fruits was estimated by thin layer chromatography (TLC) method.

13.1. Preparation of TLC plates

Silicagel (30g) containing calcium sulphate as binder was mixed with 60ml of double distilled water. The slurry was poured into a TLC spreader adjusted to a thickness of 150m and on 6 glass plates of 20x20 cm. The plates were dried in an electric oven at 100-105°C for 30 minutes and stored in a desiccator.

13.1.1. Preparation of sample

Chilli fruits with natural infection of *A. alternata* were collected, oven dried and powdered. Dry powder of chilli (20 g) was used. Apparently healthy fruits were selected for control centrifuged for 5 minutes and the optical density of the remaining mixture was read on a spectrophotometer at 720nm.

13.1.2. Preparation of standard graph

To determine the amount of capsaicin, the spot from standard graph on concentration of pure vanillin against optical density was multiplied by a factor 2 to correct the difference in the molecular weight of capsaicin and vanillin. Different volume of a standard aqueous solution of vanillin reacted with Folin-Denis reagent and optical density was determined. A standard graph was drawn with the weight of vanillin against optical density.

Percentage of capsaicin in oleoresin was calculated as :

$$\{(A \times 100)/(10^6 \times 10 \times 0.01)\} \times 100 \text{ where,}$$

A = Weight of capsaicin (Vanillin \times^2) in the spot

14. Estimation of carotene

In a flask, two gram of dry powder of red fruit was placed and 50ml saturated n-butyl alcohol was added by a pipette and filtered through what man filter paper No. 42.

The optical density of filtrate was measured at 435.8 nm in spectrophotometer. The content of carotene was calculated and expressed in ppm.

$$C = (5 \times \text{absorbance}) / (b \times K)$$

where,

C = Pigment fo carotene

b = Cell thickness

K=Constant 0.16632 absorbativity (mg/lit.) for carotene
at 435.8 nm in water saturated n-butyl alcohol.

15. Ascorbic acid estimation

One gram of fresh fruit was taken, sample was blended with 3% metaphosphoric acid (HPO_3) and *volume* was made upto 10ml with metaphosphoric acid dilution and filtered. One ml of aliquot was taken and titrated with dye solution till the change in colour from pink to colourless was there for 15 seconds. Ascorbic acid content was calculated as :

$$\{(\text{Titration reading} \times 25 \times 100) / (\text{Wt. of sample} \times 2.4 \times \text{Wt. of extract})\} \times 100$$

16. Influence of *A. alternata* on growth parame-ters

Effect of fungal infection on various growth paraneters of chilli plant was determined in four commonly available and grown varieties to know the extent of losses caused by *A. alternata*. Observations were recorded on the crop grown at IGFRI, Jhansi.

Observations on the number of fruits per plant, fruit length, fruit girth, fresh weight, plant height and yield were recorded in healthy and naturally infected chilli plants.

16.1. Chilli plant height

Height of normal healthy and diseased plants was measured from base to the top of the main stem with the help of a thread and then on the meter scale. The observations were recorded on 25 randomly selected healthy and diseased plants at 130 and 160 days.

16.2. Number of fruits per plant

Since the harvesting of chilli is irregular, the number of fruits per plant was calculated by adding the total number of fruits after four pickings of selected plants and dividing the summation with four.

16.3. Fruit length and girth

The length and girth of the diseased and healthy fruits was calculated by a thread from the joint of the calyx to the apex and then measuring it on a scale. Data were recorded on 25 fruits of each variety. Similarly, the girth was recorded.

16.4. Fresh weight

Weight of 25 healthy and diseased fruits was recorded.

17. Management of the disease:

17.1. Host resistance

Twenty-one varieties/lines of chilli were tested against fruit rot and twig infection due to *A. alternata* under natural conditions of IGFRI, Jhansi. Twenty plants of each variety were selected randomly, tagged and disease incidence was recorded on leaves, fruits and stem. Following grading was done :

	Numerical value	Description
0	Healthy	
1	Leaf/fruit/twig	Covered upto 5%
2	Leaf/fruit/twig	Covered upto 6-25%
3	Leaf/fruit/twig	Covered upto 26-50%
4	Leaf/fruit/twig	Covered upto 51-100%

The disease index was calculated by using the following formula :

Disease Index = (Sum of numerical values/ Total No. of observations) x (100/4)

Disease Index	Disease Reaction
0	Disease Free (I)
1-5	Resistant (R)
6-25	Moderately Resistant (MR)
26-50	Susceptible (S)
51-100	Highly Susceptible (HS)

17.2. Evaluation of fungicides under laboratory conditions

Following fungicides in different concentrations were tried against

A. alternata:

Sl.N.	Fungicide	Chemical Name
1.	Indofil M-45	Zinc ions+manganese ethylenebisdithio - carbamate
2.	Indofil Z-78	Zinc ethylenebidithiocarbamate
3.	Fytolon	Copper oxychloride
4.	Difolatan	N-1,1,2,2 Tetrachloro ethylthio-cis -4-cyclohexane -1, 2-dicorboximide
5.	Triforin	N,N-bis (1-formamido-2,2,2-trichloro ethyl) – piperazine
6.	Captan	N-Trichloromethylthio -4-cyclohexane 1, 2dicarboximde
7.	Thiram	Tetramethyl –thiram disulphide
8.	Derosal	Methyl-2-Benzimidazole Carbae (Carbendazim)

17.2.1 *In vitro* evaluation

Poisoned food technique was used for the evaluation of eight commonly used fungicides. Fungicides were incorporated in PDA medium after sterilization and poured into presterilised petridishes. After solidification, 5mm disc of pure culture of *A. alternata* was placed in the centre of the poisoned medium. Petridishes were incubated at $25 \pm 1^{\circ}\text{C}$. Observations were recorded on the radial growth and colony diameter after 5 and 8 day of incubation. In control, no fungicide was mixed with PDA. The discs were placed inverted to make contact of fungus with the medium.

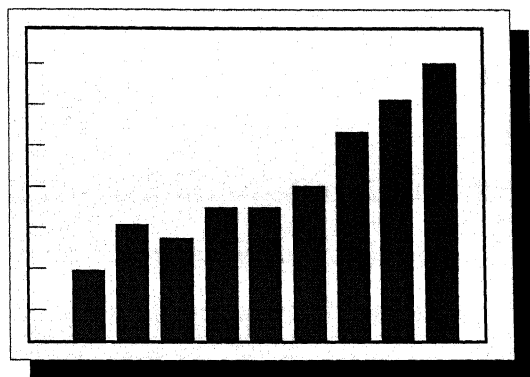
17.2.2 Seed Treatment

Management of chilli disease incided by *Alternaria alternata* was tried by seed dressing fungicides. Seed Sample No. 27 obtained from *Pandurna* was used. The sample had maximum natural infection (31.0%) of the fungus as detected by Standard Blotter Method. Observations were recorded on the associated *A. alternata* after 8days of incubation.

17.2.3. Fungicidal spray Trial

IGFRI, Jhansi form Five fungicides, Indofil M-45 (0.25%), Indofil Z-78 (0.25%), Fytelon (0.25%), Difolaton (0.25%) and Triforin (0.15%) were used as spray. Observations were recorded after three sprays starting from flowering to ripening and incidence of the fruit rot was recorded.

EXPERIMENTAL RESULTS



RESULTS

1. Survey of major diseases of chilli around Banda

In 2002 and 2003 five locations covering ten fields at each location with ten randomly selected points having at least 10 chilli plants were visited and observed for the prevalence of diseases during October to February. Data presented in table 1 indicate that at five locations around Banda and 10 major diseases were recorded. The major disease problem was the wilt incited by *Pseudomonas solanacearum* and *Fusarium spp.* Maximum wilt problem was recorded at Bisandagram on Singhpur road (42.5% plant infection) while minimum at Mavai (15.2%) on Kanpur Road. Fruit rot and leaf spot incited by *Colletotrichum sp.* ranged from 11.0 to 19.1% at Mavai and Goyara, Respectively, whereas the leaf spot problem incited by *Cercospora sp.* was maximum (12.1%) at Goyara and minimum (9.2%) at Jaspura. The mosaic disease ranged from 6.2 to 13.0% at Mavai and Bisandagram, respectively. *Sclerotinia* wilt and powdery mildew were in traces at Jaspura and Bisanda gram respectively. Other diseases like little leaf, *Phytophthora* blight and root-knot were of minor importance in 2002-03.

Table 1. Distribution of major diseases of chilli around Banda during 2002 and 2003.

S. N.	Name of the disease	Per cent diseases incidence/location					
		Jaspara	Bisandagram	Mavai	Goyara	Tindwara	Average
1.	<i>Wilt</i>	29.1	42.5	15.2	19.1	22.1	25.6
2.	<i>Little leaf</i>	5.2	7.5	3.5	1.5	3.2	4.18
3.	<i>Alternaria/</i> (LS/FR)	16.0	19.5	13.5	16.0	8.5	14.7
4.	<i>Colletotrichum/</i> (LS/FR)	12.8	17.1	11.0	19.1	17.1	15.42
5.	<i>Cercospora/LS</i>	9.2	11.2	10.5	12.1	11.5	10.9
6.	<i>Powdery mildew</i>	T	T	3.0	6.5	3.2	2.7
7.	<i>Phytophthora</i> <i>blight</i>	4.6	6.7	4.5	T	T	3.1
8.	<i>Sclerotinia wilt</i>	T	T	10.5	8.4	7.2	5.22
9.	<i>Mosaic</i>	11.4	13.0	6.2	7.9	7.0	9.1
10.	<i>Root knot</i>	6.5	7.2	7.1	3.2	4.0	5.6

Mean of two year observation

T = Traces less than 1.0%

Fruit rot and leaf spot incited by *Alternaria alternata* was observed in all five locations and ranged from 8.5 to 19.5% at Tindwara and Bisanda gram, respectively. The *Alternaria* fruit rot and leaf spot was considered to be the major problem and ranked third to wilt and *Colletotrichum* sp. Under Banda conditions very little is known about *Alternaria* fruit rot and leaf spot.

1.1. Survey of *Alternaria* fruit rot in and around Banda and Jhansi.

1.1.1. Banda

During 2002, 2003, and 2004 around Banda 20 locations were visited for the occurrence of fruit rot of chillies during September–February. Data presented in Table 2 indicate that the fruit rot and twig drying disease 16.6 percent (in 2002), 18.2% (in 2003) and 20.0% (in 2004) was at Para and Jari, respectively, with maximum average (18.0%)

also at Jari. In 2002 maximum (27.1%) fruit rot was recorded at Newada, whereas it was maximum (24.0%) at Baberu and Barokhar 2003 and 2004, respectively. On the basis of average of three years data, maximum fruit rot (21.4%) was recorded at Kanwara and Baberu, while maximum twig drying (18.0%) was recorded at the vegetable gardens of Jari (Table 2).

Table 2. Incidence of twig drying and fruit rot of chilles incited by *Alternaria alternata* around Banda during 2002, 2003 and 2004.

S. N.	Location	% twig drying			Average	% fruit rot			Average	Cropping Pattern
		2002	2003	2004		2002	2003	2004		
1	2	3	4	5	6	7	8	9	10	11
1.	Mavai	4.8	9.3	12.6	8.9	12.0	14.1	15.6	13.9	Sole+Mixed
2.	Para	16.6	12.5	19.2	16.1	11.2	21.3	17.3	16.6	Sole+Mixed
3.	Bisanda gram	6.7	11.6	11.1	9.8	8.5	11.3	11.7	10.5	Sole+Mixed
4.	Jaspura	10.0	14.6	10.2	11.6	13.1	16.4	8.3	12.6	Sole+Mixed
5.	Tindwara	9.5	6.5	9.5	8.5	7.2	19.2	8.4	11.6	Mixed
6.	Jamrehi	7.5	8.5	8.3	8.1	8.3	8.2	17.1	11.2	Mixed
7.	Goyara	11.0	12.6	17.2	13.6	17.6	20.1	15.4	17.7	Sole+Mixed
8.	Koni	10.4	18.0	16.3	14.0	8.2	19.2	18.2	15.2	Mixed
9.	Sabada	5.2	4.6	11.5	7.1	17.2	5.7	11.6	11.5	Mixed
10.	Newada	7.8	12.7	13.7	11.4	27.1	17.2	18.7	21.0	Sole
11.	Belganva	2.6	12.5	13.5	9.8	24.5	12.5	17.5	18.0	Mixed
12.	Pangara	4.0	6.0	9.0	7.0	5.4	11.0	13.0	10.0	Mixed
13.	Palhari	6.0	7.0	4.8	6.0	18.0	16.2	14.4	16.5	Mixed
14.	Kanwara	9.5	7.2	8.0	8.0	23.0	22.0	14.3	21.4	Mixed
15.	Ghoori	6.7	14.1	17.5	12.4	21.5	18.0	17.0	19.5	Mixed
16.	Jaurahi	16.0	17.5	6.5	12.5	25.2	17.0	18.9	20.0	Sole
17.	Jari	14.5	18.2	20.0	18.0	17.4	15.9	13.0	15.0	Mixed
18.	Baberu	3.0	9.5	9.5	7.4	19.0	24.0	22.8	21.4	Sole+Mixed
19.	Singhpur	1.5	8.5	3.5	4.5	18.7	11.0	18.5	15.6	Mixed
20.	Barokhar	13.5	9.6	4.3	9.0	14.0	11.0	24.0	16.0	Mixed

It was interesting to note that cropping pattern had no significant influence on the disease incidence, mixed and sole cropping system of chillii exhibited no variation (Table 2).

1.1.2.Jhansi

During 2003 and 2004 around Jhansi 15 locations were visited for the occurrence of fruit rot incited by *Alternaria sp.* during October-February and the results are presented in Table 3.

Data presented in Table 3 indicate that the fruit rot and twig drying disease incited by *Alternaria sp.* was present in all the 15 locations during 2003 and 2004. Maximum twig drying was recorded at Hansari (17.2%) and Chirgaon (17.2%) in 2003 and 2004, respectively, whereas minimum disease (3.8%) and 7.0% was recorded at Sakrar and Bhagwantpura in 2003 and 2004 respectively on the other hand, maximum fruit rot of 23.3% was recorded at Karari and Khailar village respectively in 2003, whereas it was 27.4% at Batta village in 2004. In 2003 and 2004 minimum fruit rot problem was 12.4 and 14.4 % at Talbehat and Lahargird villages, respectively . It is clear from the data presented in Table 3, that twig drying and fruit rot incited by *Alternaria alternata* is a wide spread problem being observed maximum (on the average basis of two years) at Chirgaon (16.2%) and Khailar (23.2%), respectively.

On the basis of data presented in Table 2 and 3, it is concluded that the tender tip drying and fruit rot of chillies incited by *Alternaria alternata* is a wide spread problem in Banda and Jhansi.

Table 3. Incidence of twig drying and fruit rot of chillies around Jhansi during 2003 and 2004

S.	Location	% twig drying		Average	% fruit rot		Average
N.		2003	2004		2003	2004	
1	2	3	4	5	6	7	8
1.	Chirgaov	15.0	17.2	16.2	21.0	19.2	20.1
2.	Baraganv	7.0	9.0	8.0	19.2	18.0	18.6
3.	Parichha	8.4	7.6	8.0	17.8	19.0	18.4
4.	Bhagwantpura	7.0	7.0	7.0	19.0	21.4	20.2
5.	Bijauli	11.0	11.3	11.15	22.2	23.4	22.8
6.	Khailar	11.3	14.5	12.9	23.3	23.3	23.2
7.	Lahargird	13.2	12.2	12.7	17.6	14.4	16.0
8.	Batta	5.4	7.2	6.3	18.4	27.4	22.9
9.	Sakrar	3.8	9.2	6.5	19.8	16.4	18.1
10.	Baruwasagar	17.1	16.2	16.5	20.0	18.1	19.5
11.	Babeena	10.4	12.8	11.6	13.4	21.4	17.4
12.	Bansi	10.4	15.0	12.7	13.4	19.3	16.35
13.	Talbehath	15.0	11.2	13.1	12.4	16.2	14.3
14.	Hansari	17.2	12.4	14.8	21.2	17.4	19.3
15.	Karari	10.0	11.4	10.7	23.3	16.0	19.65

1.1.3. Incidence of fruit rot of chilli incited by *Alternaria alternata* observed during October and January of 2002 and 2003

At five locations around Banda, observations on the incidence of fruit rot was recorded in 2002 and 2003. The data are presented in Table 4. It is clear from the data that fruit rot was present in both the seasons in both the years. During 2002 maximum 16.2% and 23.1% fruit rot was recorded during October and January, respectively at Jaspura, while in 2003 maximum 14.2% fruit rot (at Mavai) and 23.4% (at Bisandagram) was recorded during October and January, respectively. It is also clear

from the data that the fruit rot was more severe during January /February as compared to September/October (Table 4) in almost all the five locations.

Table 4. Incidence of fruit rot of chilli in the months of September/October and January/February observed at Banda during 2002 and 2003

S. N.	Name of the Location	% Fruit rot					
		2002		2003		Average	
		Sept/Oc t	Jan/Fe b	Sept/Oc t	Jan/Fe b	Sept/Oc t	Jan/Fe b
1.	Jaspura	16.2	23.1	13.0	19.3	14.6	21.2
2.	Bisanda gram	12.0	19.1	17.4	23.4	14.7	21.3
3.	Mavai	9.2	15.8	14.2	16.6	11.7	16.2
4.	Goyara	13.4	17.1	11.4	17.5	12.4	17.3
5.	Tindawara	11.0	18.0	9.0	14.0	10.0	16.0
Average						12.7	18.6

2. Collection of Samples

2.1. Plant parts

Infected plants of chillies were collected and were cut into small pieces and parts viz. root lets, roots, collar region, stem, leaves and fruits were kept in paper envelops and brought to the laboratory for further studies.

2.2. Seed samples

Chilli seed samples of 19 varieties were obtained from the Scientist, Department of Horticulture, CSA, University of Agriculture and Technology, Kanpur, and 20 seed samples were collected from 20 locations and 11 samples of chilli seeds were collected from the IVRI, Varansi. Therefore, in all 50 chilli seed samples were used in the present

study. The seed samples were numbered and stored in paper envelopes at low temperature (4°C) to avoid any deterioration.

3. Isolation, purification and identification of the fungus

3.1. Diseased chilli plant parts were brought to the laboratory and isolations were made on potato-dextrose-agar medium in Petridishes under aseptic conditions. On repeated isolations *Alternaria alternata* was found constantly associated with infected fruits, twig and leaves. The fungus was also found associated with chilli seeds when incubated on moist blotters and PDA medium. No variation in different isolates was recorded.

3.1. Purification of the culture

The culture of *Alternaria alternata* was purified by first fungal hyphal tip method, thereafter by single spore isolation method (Ezekiel, 1930).

3.3.1. Cultural characters

The mycelium was aerial, abundant varying from fluffy, cottony to closely tufted. The pigmentation was grayish, olive green and almost black in old cultures.

3.3.2. Mycelium

The hyphae were branched pale brown closely septate, 3-4 μ in thickness.

3.3.3. Conidiophores

They were formed somewhat aggregated in tufts or sometimes evenly distributed on the mycelium. They were simple or branched, erect olive brown, septate, geniculate and often with several scars and swellings.

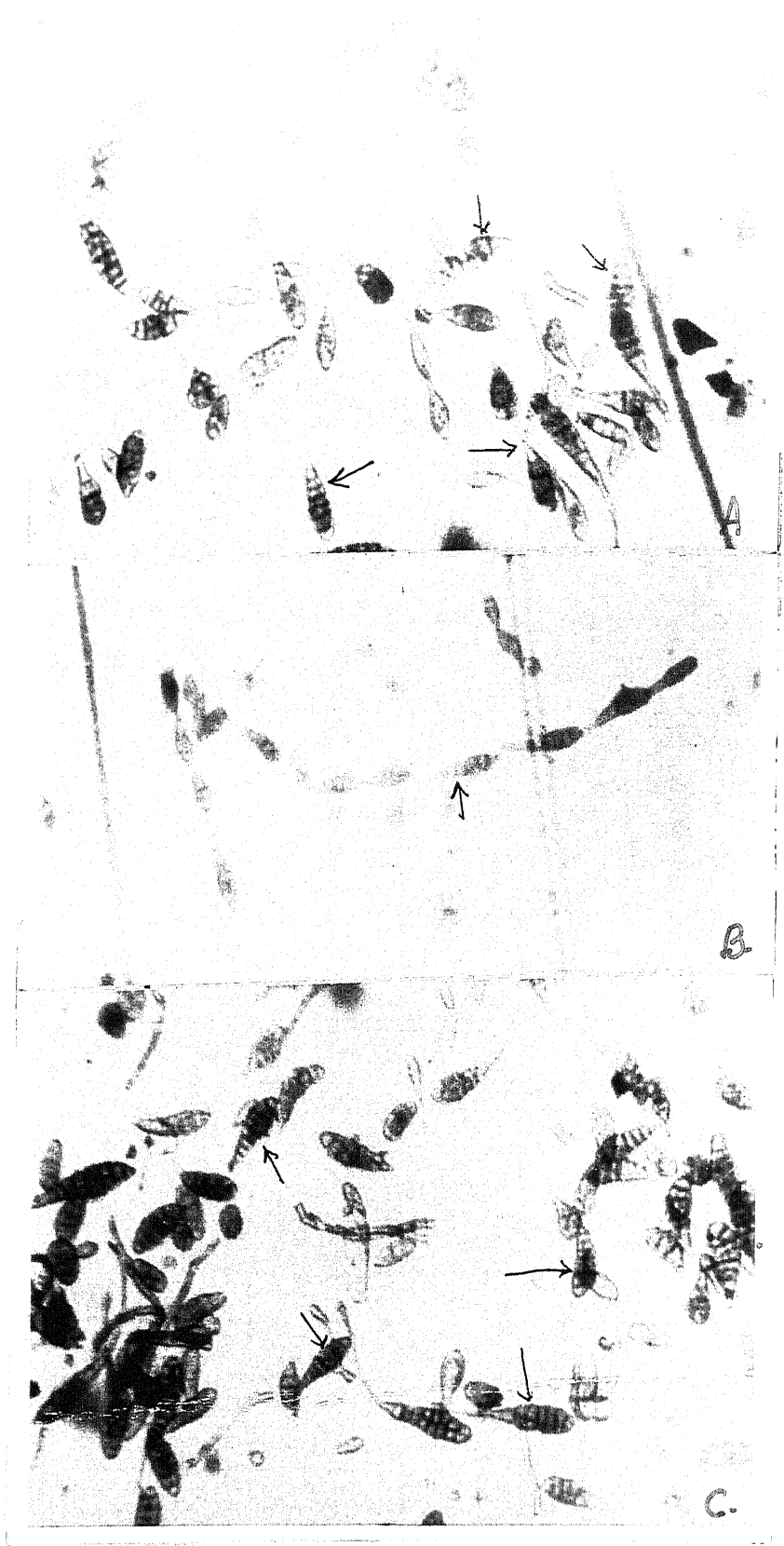


Fig.1. A) Conidia of *Alternaria alternata*.
 B) Chains of conidia
 C) Conidia variable in size with beak and muriform.



Fig.2. Chilli seed infected with *Alternaria alternata*.

3.3.4. Conidia

The conidia were light olive brown to dark brown, smooth, muriform with 3 to 6 transverse septa and 1 to 3 longitudinal septa. The conidia were variable in shape and size ranging from 13.2-42.8 x 10.6 to 13.2 μ . The conidia were in chains. The size of the beak ranged from 10-20 x 2-6 μ .

The characters of the isolated fungus confirmed it to be *Alternaria alternata* (Fr.) Keissler (Fig. 1).

4. Symptomatology

Symptoms incited by the fungus on chilli seeds, seedlings, leaves, twigs and fruits were recorded.

4.1. On seeds

Infected chilli seeds were smaller in size, rusty brown to black and in severe cases shrinkage was recorded. In badly infected fruits the seeds just beneath the pericarp were black and full of mycelium. Infected seeds did not germinate and rotted (Fig. 2).

4.2. On seedlings

Seedlings of 10 days showed pale brownish, sunken discolouration of young stem above the collar region. Seedlings collapsed and toppled down. In severe cases the grayish mycelium was observed on the infected portions.

4.3. On stem and twigs

Necrosis of tender tip of chilli plants from the tip backwards was recorded in the month of September/January. The diseased portion become thinner in comparison to healthy portion because of drying. A brown black irregular demarcation line separated the infected portion from healthy one.



A



B



C

Fig.3.

- i) A) Infected chilli plants.
- B) Infected fruits of chilli.
- C) Necrosis of tender tips of chilli plant exhibited "White wash".

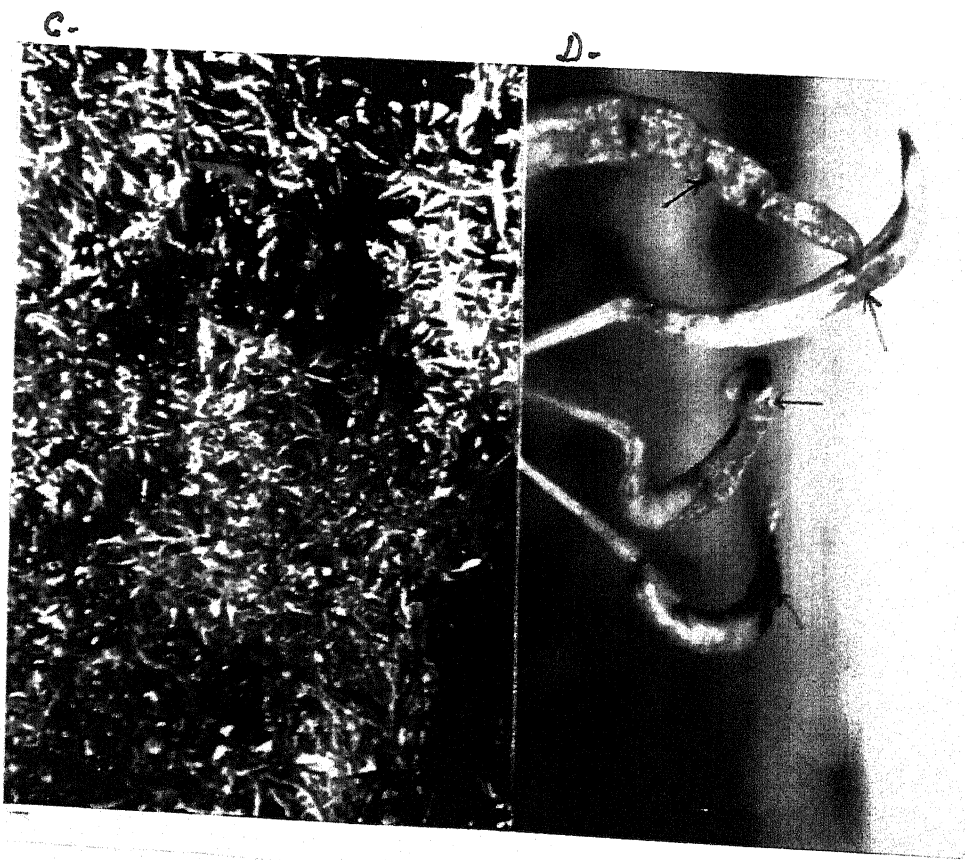
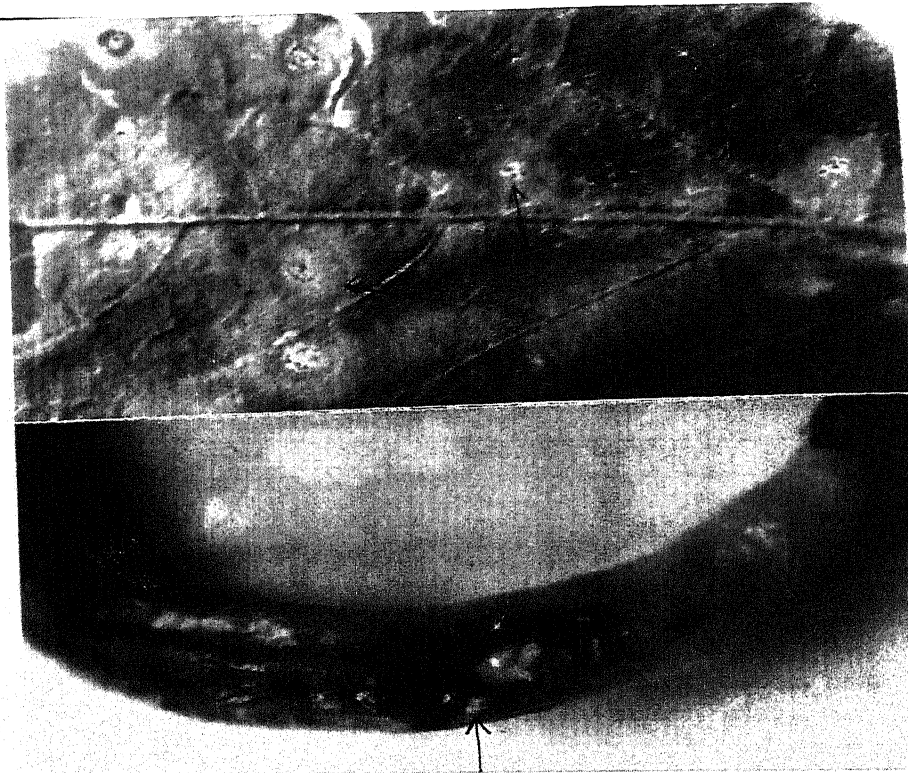


Fig.3. ii) Heavily infected field and fruits of chilli crop.



A



B

C

Fig.4. A), B) Development of concentric ring on leaf.
C) Symptoms of fruit rot of chilli.

Under field conditions, this phase could easily be identified by workers. In severe conditions the tender tip drying is very conspicuous and can be distinguished on the basis of "white wash" symptom. The tips become silvery white. On the stem, under severe conditions, irregular brown specks were common (Fig. 3(i)).

4.4. On leaves

Leaf spots incited by the fungus were observed. At initial stage, the lesions were small, isolated, scattered pale brown. Lower sensitive leaves were usually attacked first and disease progressed upwards and made the affected leaves to go into yellowish, ultimately the leaves dried up and dropped off. In the necrotic tissues concentric rings developed to produce target-board effect. There was a narrow chlorotic zone around the spots which faded into normal green and increased with the increase in size of spots. Infection from the stem spread to petiole. The petiole developed brown to black discolouration (Fig. 4).

4.5. On fruits

Fruit rot and spots were observed. On semi red fruits of chilli, small brown black spots appeared which later advanced and covered the entire fruit. The pericarp of the infected fruit became greenish brown. The fungus also attacked the ripe fruits. Irregular concentric spots were also observed. In advanced fruit rot conditions, the seeds just beneath the pericarp were also found infected and covered with mycelium. Infected seeds turned black brown. The affected fruits developed large, greenish brown to brown specks/lesions. Later on, they were covered with a dark grayish brown to black velvety moldy growth of the fungus (Fig. 3(i) & Fig. 4).



Fig.5. Isolation of *A. alternata* from deferent plant parts.

5. Association of *Alternaria alternata* with chilli plant parts

Different infected plant parts of chili were subjected for isolation on PDA. Observations were recorded on the eight day of incubation and results are presented in Table 5.

It is evident from the data presented in Table 5 that the fungus was associated with stem, leaf and fruit parts, while the association of *A. Alternata* was not observed with chilli roots, rootlets and flowers. Maximum (38.0 per cent) association was observed in leaf lamina, followed by 36.0 per cent with seeds. The fungus was predominatly with seeds, pericarp, fruit stalk, leaf lamina and tender twigs (Fig. 5).

Table 5. Association of *Alternaris alternata* with chilli plant parts isolated on potato dextrose agar medium

Plant part		Number of isolations made	Presence/Absence	% association
Root	Rootlets	50	Absent	0.0
	Root	50	Absent	0.0
Stem	Collar region	50	Present	15.0
	Stem	50	Present	10.0
	Tender twig	120	Present	17.0
Leaf	Petiole	100	Present	16.0
	Lamina	400	Present	38.0
Flower	Flower	100	Absent	0.0
Fruit	Fruit stalk	450	Present	18.5
	Fruit skin	450	Present	24.0
	Placenta	400	Present	23.0
	Seed	400	Present	36.0

6. Pathogenicity Tests

The virulence of isolated *A. alternata* was tested by three methods and the data are presented in Table 6 and 7.

6.1. Standardization of time required for maximum conidial production of *Alternaria alternata*

For maximum conidial production, the time required was determined by making conidial count on 6, 8, 10, 12, 14, 16 and 18 days interval. The data on conidial count per 0.1 ml suspension exhibited that number of conidia increased with the duration of incubation period upto 8th day and later on there was a gradual decline.

6.2. Seed infestation method

Chilli seeds free from natural infection of *Alternaria alternata* collected from Kanpur road, Banda were rolled on actively grown culture in Petridish, after soaking in sterile water for 120 minutes. Seeds infested with culture were placed on moist blotters in Petridish and were incubated in growth chamber. The observations were recorded on seed infection due to the test fungus and the results are presented in Tabale 6.

It is evident from the data presented in Table 6 that in case of injury made on chilli seeds, comparatively greater infection could be observed as against the uninjured seeds. Injuries were made with the help of sterile entomological needles. Out of 100 seeds, 63 chilli seeds exhibited the infection when injury was made as compared to 45.0% in uninjured seeds. The germination was 61.0% as compared to uninjured seeds having only 63.0% germination. In control, 83% and 73% seed germination was recorded in uninjured and injured seeds. The injured seeds showed more infection and less germination, whereas uninjured seeds had higher germination and lesser infection (Table 6). In control, on infection was recorded as the seeds were neither infested not had natural infection.

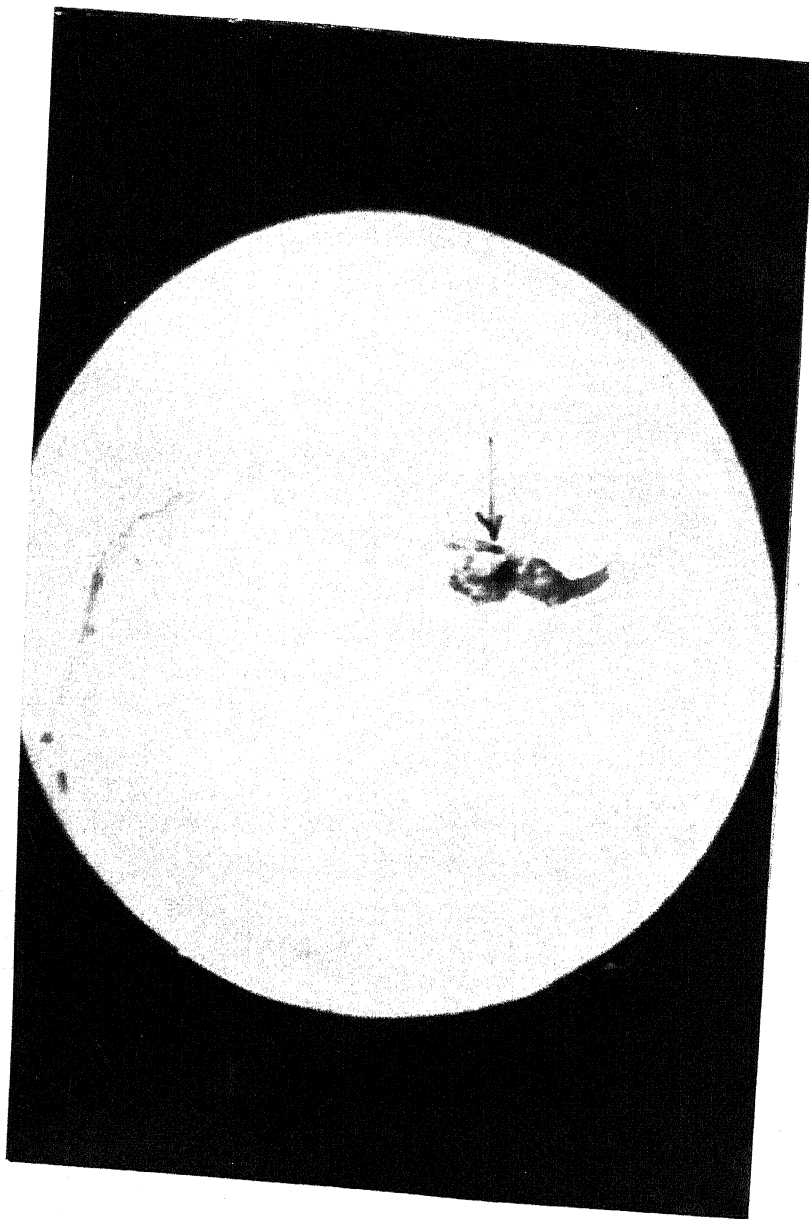


Fig.6. Effect of fruit inoculation on chilli.

6.4. Fruit inoculation method

The virulence of the isolated *A. alternata* was tested on the fruits and leaves of Pusa Jwala, a commonly used variety around Banda by four methods-pin prick, *Alternaria* spore suspension spray, carborandum rub and tooth prick. The observations were recorded on the symptom expression (Fig. 6). The data are presented in Table 7.

Semi ripe and ripe fruits and leaves of Pusa Jalwa were inoculated and kept under belljar in laboratory conditions with high humidity. It is clear from the data (Table 7) that in detached leaves the disease symptoms appeared in 6, 11 and 7 days of pin prick, spore suspension spray and carborandum rub methods, respectively. In semi ripe fruits when the mycelia bit was introduced with the help of tooth prick, the disease symptoms appeared within 10 days in detached fruits and 12 days in intact fruits, whereas the symptoms appeared in 8 and 10 days in pin prick method, respectively. Although the symptoms appeared within 5 and 7 days in carborandum rub method but due to large necrotic areas the typical symptoms were not observed. In case of ripe fruits, disease symptoms appeared comparatively earlier than semi ripe fruits. Here also pin prick method was better as within 7 and 9 days typical symptoms appeared on intact and detached fruits. Spore suspension of *Alternaria alternata* when sprayed with an automizer the symptoms appeared within 15 and 13 days on fruits. In semi ripe and ripe fruits pin prick method was found to be the best as the symptoms appeared within 7 to 10 days (Table 7).

Similarly on detached and intact leaves pin prick method was the best and typical symptoms were recorded on 6th and 8th day, respectively. Although the infection took place within 6 and 7 days in leaves but due to large necrotic patches the carborandum rub method could not be considered as best. In spore suspension spray method about 12 days were

required to produce symptoms on intact leaves and 11 days for detached leaves.

It was concluded that pin prick method was the best to test the virulence of *A. alternata* on chilli leaves, semi ripe fruits, and ripe fruits (Table 7).

Table 7. Efficacy of four methods for testing pathogenicity of *Alternaria alternata* on leaves and fruits of Pusa Jwala

Method	Symptom expressed after days					
	Leaves		Semi ripe fruit		Ripe fruit	
	Intact	Detached	Intact	Detached	Intact	Detached
Pin prick	8	6	10	8	7	9
Spore suspension spray	12	11	13/15	13	15	13
Carborandum rub	6*	7*	7*	5*	5*	4/5*
Tooth prick	-	-	12	10	9	11

* Large necrotic area formed (-) Not done

7. Detection of mycoflora with chilli seeds

In all 50 seed samples of chilli were collected and tested for the associated *A. alternata* and other mycoflora by Standard Blotter Method (ISTA, 1985). The samples were grouped into two – (i) Samples locality-wise, and (ii) samples variety-wise.

Table 8, Fungi associated with 31 seeds samples of chillies collected from different place as detected by standard Blotter method

Mycoflora	Seed sample/% Mycoflora associated															
	1		2		3		4		5		6		7		8	
	U	P	U	P	U	P	U	P	U	P	U	P	U	P	U	P
<i>Alternaria alternata</i>	12	12	8	8	12	12	16	12	11	8	7	2	2	4	14	12
<i>Alternaria sp.</i>	8	4	0	2	2	3	8	2	8	3	12	3	4	0	4	2
<i>Aspergillus flavus</i>	2	0	5	2	3	0	8	3	4	1	0	1	3	0	1	0
<i>Aspergillus niger</i>	1	0	0	0	2	0	0	3	3	0	1	2	3	0	2	0
<i>Aspergillus fumigatus</i>	0	0	5	0	1	1	2	0	3	3	0	1	2	0	3	0
<i>Botrytis cinerea</i>	2	0	2	0	8	0	1	2	3	0	0	1	6	0	0	0
<i>Curvularia lunata</i>	13	8	2	1	0	0	1	0	0	0	13	2	0	0	0	0
<i>Curvularia sp.</i>	2	0	0	0	1	0	2	0	0	0	2	0	4	0	0	0
<i>Chaetomium sp.</i>	4	4	1	0	0	0	0	0	0	2	0	2	0	0	0	0
<i>Cladosporium oxysporum</i>	3	3	1	0	4	2	0	0	4	3	9	0	4	3	5	4
<i>Colletotrichum capsici</i>	4	3	8	4	11	8	4	3	4	0	14	10	9	6	12	9
<i>Drechslera tetramera</i>	1	0	4	0	0	0	1	0	6	0	0	0	12	0	1	0
<i>Fusarium oxysporum</i>	2	2	0	0	8	0	2	0	0	0	0	0	4	0	6	0
<i>Fusarium solani</i>	0	1	6	0	0	1	0	0	0	0	2	0	0	0	6	0
<i>Memnoniella sp.</i>	0	0	8	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Penicillium sp.</i>	2	0	2	0	9	0	1	0	2	0	8	0	4	0	2	0
<i>Rhizopus sp.</i>	0	0	0	1	1	2	0	0	0	0	0	0	3	0	2	0
<i>Total frequency</i>	56	37	52	19	62	29	46	25	48	20	68	24	60	13	58	22
<i>% germination</i>	46	67	64	71	40	72	49	64	50	69	34	70	41	49	32	68

1. Urai

2. Jalaun

3. Lalitpur

4. Kanpur

5. Kannauj

6. Mainpuri

7. Etawah

8. Etah

Table 8 (Contd.)

Mycoflora	Seed sample/% Mycoflora associated															
	9		10		11		12		13		14		15		16	
	U	P	U	P	U	P	U	P	U	P	U	P	U	P	U	P
<i>Alternaria alternata</i>	19	14	16	14	6	4	16	18	22	20	10	9	22	21	22	20
<i>Alternaria sp.</i>	0	0	4	3	10	10	0	0	0	10	4	4	0	0	0	0
<i>Aspergillus flavus</i>	2	0	3	1	0	2	14	2	10	0	15	3	4	0	6	0
<i>Aspergillus niger</i>	5	0	0	0	0	3	11	0	14	0	0	0	3	0	18	11
<i>Aspergillus fumigatus</i>	0	0	2	2	0	0	4	0	8	0	0	0	0	0	0	0
<i>Botrytis cinerea</i>	3	1	6	0	2	0	8	0	4	0	0	0	6	0	11	0
<i>Curvularia lunata</i>	0	0	2	3	3	0	7	8	3	0	4	0	5	0	0	0
<i>Curvularia sp.</i>	12	4	0	0	0	0	0	2	0	0	10	0	0	0	4	0
<i>Chaetomium sp.</i>	2	0	1	0	3	0	0	0	3	0	2	0	2	0	1	4
<i>Cladosporium oxysporum</i>	6	1	2	1	3	0	0	0	6	0	4	0	0	0	2	3
<i>Colletotrichum capsici</i>	10	8	20	19	23	20	9	10	10	12	8	6	8	9	16	12
<i>Drechslera tetramera</i>	0	0	3	0	0	0	2	1	4	0	6	0	2	0	1	0
<i>Fusarium oxysporum</i>	11	0	5	0	4	0	1	0	0	0	2	2	6	0	2	0
<i>Fusarium solani</i>	3	0	2	0	8	0	0	0	0	0	1	0	7	0	4	0
<i>Memnoniella sp.</i>	0	0	0	0	9	0	0	0	0	0	10	2	0	0	0	0
<i>Penicillium sp.</i>	0	0	1	0	3	1	0	0	1	0	4	0	1	0	2	2
<i>Rhizopus sp.</i>	1	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0
Total frequency	74	28	67	43	78	41	72	41	85	42	80	26	66	30	88	52
% germination	44	44	52	58	63	49	58	57	21	54	30	68	16	63	20	48

9. Hardoi

10. Lakhimpur

11. Sitapur

12. Pilibhit

13. Shahjanhapur

14. Bareilly

15. Fatehpur

16. Allahabad

Table 8 (Contd.)

Mycoflora	Seed sample/% Mycoflora associated															
	17		18		19		20		21		22		23		24	
	U	P	U	P	U	P	U	P	U	P	U	P	U	P	U	P
<i>Alternaria alternata</i>	18	16	6	8	0	0	11	10	28	20	11	11	14	13	28	24
<i>Alternaria sp.</i>	4	1	10	4	0	0	0	0	2	4	0	0	1	0	3	0
<i>Aspergillus flavus</i>	4	2	8	2	10	1	12	0	2	0	0	0	0	0	0	1
<i>Aspergillus niger</i>	2	0	4	0	3	0	1	0	1	0	2	0	2	0	4	3
<i>Aspergillus fumigatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Botrytis cinerea</i>	4	2	1	0	3	2	6	4	1	0	0	0	0	0	2	1
<i>Curvularia lunata</i>	6	0	6	0	3	0	4	0	3	0	1	0	0	0	3	0
<i>Curvularia sp.</i>	5	0	4	2	3	0	2	0	4	0	1	0	0	0	0	0
<i>Chaetomium sp.</i>	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	2
<i>Cladosporium oxysporum</i>	2	0	3	1	4	0	0	0	2	0	0	0	1	0	0	0
<i>Colletotrichum capsici</i>	12	11	16	10	8	10	8	12	14	10	22	11	0	0	8	2
<i>Drechslera tetramera</i>	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fusarium oxysporum</i>	0	0	0	0	2	2	4	0	4	2	0	0	0	0	0	1
<i>Fusarium solani</i>	0	0	0	0	1	0	6	0	0	0	0	0	0	0	6	2
<i>Memnoniella sp.</i>	4	0	0	0	2	0	4	0	0	0	0	0	2	0	0	0
<i>Penicillium sp.</i>	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
<i>Rhizopus sp.</i>	2	0	1	0	0	0	0	0	2	0	0	0	1	0	1	0
Total frequency	65	37	66	27	43	15	58	26	63	36	28	22	21	13	55	36
% germination	31	50	30	49	91	91	42	69	31	66	66	77	72	68	41	42

17. Barabanki

18. Faizabad

19. Kurvi

20. Gonda

21. Jhansi

22. Deoria

23. Varanasi

24. Hamirpur

Table 8 (Contd.)

Mycoflora	Seed sample/% Mycoflora associated														
	25		26		27		28		29		30		31		Maximum %association
	U	P	U	P	U	P	U	P	U	P	U	P	U	P	
<i>Alternaria alternata</i>	0	3	20	17	30	28	26	20	16	12	32	30	20	18	32.0
<i>Alternaria sp.</i>	12	8	0	2	0	1	0	0	0	0	0	3	4	2	12.0
<i>Aspergillus flavus</i>	2	0	4	0	8	0	6	4	8	0	0	3	4	10	15.0
<i>Aspergillus niger</i>	5	2	0	0	0	0	0	0	0	4	0	2	0	0	18.0
<i>Aspergillus fumigatus</i>	3	1	0	0	0	0	0	0	0	0	0	3	0	0	8.0
<i>Botrytis cinerea</i>	8	2	8	0	1	0	2	0	1	0	2	4	0	0	11.0
<i>Curvularia lunata</i>	0	0	0	0	0	0	3	0	16	3	0	0	8	0	16.0
<i>Curvularia sp.</i>	1	0	0	0	3	2	4	0	0	0	0	0	0	2	12.0
<i>Chaetomium sp.</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4.0
<i>Cladosporium oxysporum</i>	6	0	0	1	8	8	0	0	0	0	0	0	0	0	8.0
<i>Colletotrichum capsici</i>	9	9	29	27	0	0	12	12	24	11	0	2	1	1	29.0
<i>Drechslera tetramera</i>	2	1	2	0	2	0	0	0	1	0	0	0	3	0	12.0
<i>Fusarium oxysporum</i>	4	1	3	0	0	0	0	2	6	0	0	0	4	2	11.0
<i>Fusarium solani</i>	4	3	4	2	0	2	0	0	2	0	2	2	2	0	8.0
<i>Memnoniella sp.</i>	6	0	3	0	0	0	0	0	4	0	3	0	0	0	10.0
<i>Penicillium sp.</i>	0	0	5	0	1	0	2	0	8	1	6	1	0	0	9.0
<i>Rhizopus sp.</i>	0	2	6	0	0	0	0	2	12	4	8	0	0	0	12.0
Total frequency	62	33	84	48	52	41	55	40	98	34	53	50	46	35	
% germination	42	52	30	48	51	42	60	50	20	32	52	49	44	61	

25. Mirzapur

26. Jaunpur

27. Mahoba

28. Sultanpur

29. Meerut

30. Banda

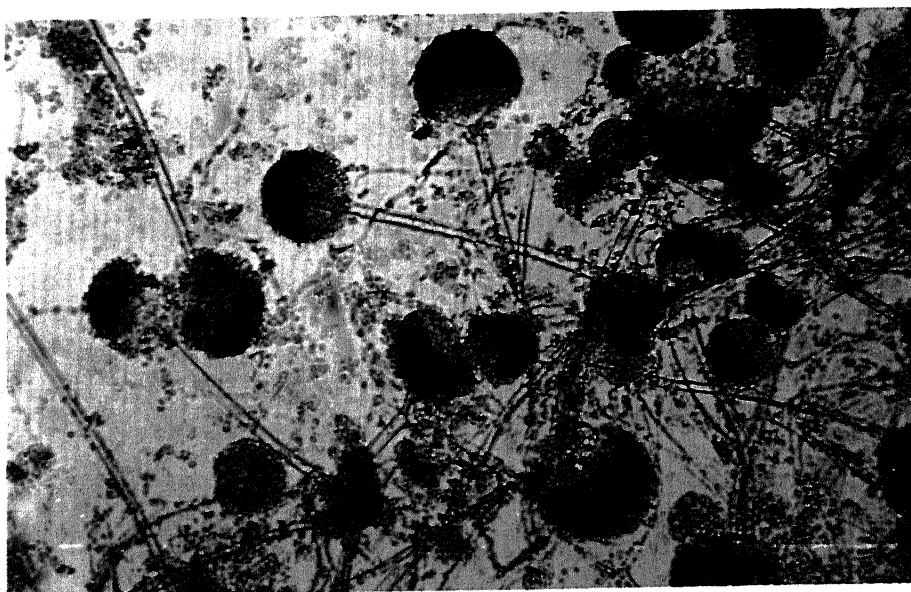
31. Bijnaor



A.



B.



C.

Fig.7. Identification of seed mycoflora
A) Penicellium, B) Rhizopus, C) Aspergillus

Overall 17 fungi were found associated with chilli seed samples. The association of *Alternaria alternata* ranged from 2.0 to 32%. The association of other fungi is as follows: *Aspergillus niger* from 1.00 to 18.00%, *Aspergillus flavus* from 1.00 to 15.00%, *Aspergillus fumigatus* from 2.00 to 8.00%, *Botrytes cinerea* from 1.00 to 11.00%, *Curvularia lunata* from 1.00 to 16.00%, *Curvularia sp.* from 1.00 to 12.00%, *Chaetomium sp.* from 1.00 to 4.00%, *Cladosporium oxysporum* from 1.00 to 8.00%, *Colletotrichum capsici* from 1.00 to 29.00%, *Drechslera tetramera* from 1.00 to 12.00%, *Fusarium oxysporum* from 1.00 to 11.00%, *Fusarium solani* from 1.00 to 8.00%, *Memnoniella sp.* from 1.00 to 10.00%, *Penicillium sp.* from 1.00 to 9.00% and *Rhizopus sp.* from 1.00 to 12.00% (Fig. 7). The association of other species or *alternaria* was maximum 11.00% (Table 8).

7.2 Variety-wise

Nineteen varieties/lines were obtained from the Department of Horticulture, CSA University of Agri. and Tech., Kanpur Seeds were tested for the association of mycoflora without pretreatment by using Standard Blotter Method (ISTA, 1985) and results are presented in Table 9.

Fourteen fungi were found associated with chilli seeds when incubated on moist blotters. Maximum number of fungi (63) was recorded on JCA 181, followed by 66 on JCA 111 and Pandurna. Least number of fungi (22) was associated with Musalwadi variety (Table 9). The germination of seeds ranged from 61.0 to 91%.

The association of *Alternaria alternata* ranged from 4.00 to 25.00%, *Aspergillus flavus* from 2.00 to 12.00%, *Aspergillus niger* from 2.00 to 10.00%, *Aspergillus fumigates* from 1.00 to 8.00%, *Botrytes cinerea* from 1.00 to 11.00%, *Curvularia sp.* from 1.0 to 6.0%, *Curvularia lunata* from 1.00 to 12.00%, *Chaetomium sp.* from 1.00 to

5.00%, *Cladosporium oxysporum* from 2.00 to 6.00%, *Colletotrichum capsici* from 1.00 to 18.00%, *Fusarium oxysporum* from 1.00 to 8.00%, *Fusarium solani* from 2.00 to 10.00%, *Penicillium sp.* from 1.00 to 5.00 per cent and *Rhizopus sp.* from 1.00 to 4.00% (Table 9).

Table 9. Fungi associated with untreated seeds of 19 Varieties of chillies as detected by Standard Blotter Method

Variety	% mycoflora associated														Total	% Germination
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Pandurna	15	4	4	0	0	0	10	3	2	12	4	3	4	2	63	61
NP 46 A	16	3	0	0	11	0	4	1	0	14	0	8	2	3	63	62
Pusa Jwala	14	4	0	2	0	2	5	4	0	8	2	10	0	4	55	68
Kaliyanpur Red	6	2	0	0	0	0	8	2	0	14	2	4	0	0	38	70
Bunchy	8	3	4	8	2	1	4	3	0	18	2	3	0	5	61	72
G-3	22	0	0	2	0	0	0	2	0	9	0	8	0	4	47	72
SG-3	25	0	0	2	0	0	12	2	0	10	0	0	0	0	51	85
C-1 Pant	22	0	2	0	0	2	12	5	0	10	0	0	0	0	53	62
JCA 20	4	10	2	0	2	2	11	4	0	0	0	0	0	1	36	74
JCA 31-8	6	0	2	3	1	0	0	0	0	0	8	0	0	2	22	80
JCA 111	22	0	6	2	2	2	0	0	0	12	6	10	0	4	66	90
JCA 154	14	0	0	1	0	0	0	0	0	4	0	8	0	1	28	81
JCA181	16	12	4	2	0	6	4	0	4	14	0	0	0	1	63	78
JCA 208	18	0	0	0	1	0	8	3	2	0	1	2	0	1	36	64
JCA 232	16	4	10	0	2	0	0	0	3	0	0	0	0	0	35	72
Jawahar Mirch 218	24	0	8	0	4	1	4	2	2	4	0	0	0	0	49	84
K2	10	11	0	0	0	0	3	0	6	12	0	0	0	0	42	91
Musalwadi	8	10	0	1	0	0	2	0	0	2	2	0	0	0	25	82
LCA 206	16	0	10	2	8	6	1	0	2	1	2	0	0	0	47	72

1. *Alternaria alternata*

2. *Aspergillus*

3. *Aspergillus niger*

4. *Aspergillus fumigates*

5. *Botrytis cinerea*

6. *Curvularia sp.*

7. *Curvularia luntata*

9. *Cladosporium*

11. *Fusarium oxysporum*

13. *Penicillium sp.*

8. *Chaetomium sp.*

10. *Colletotrichum capsici*

12. *Fusarium solani*

14. *Rhizopusasp.*

7.3. In all 50 seed samples of chilli were analysed for the association of mycoflora by Standard Blotter Method and the results are presented in Table 8 and 9. On the basis of maximum natural infection of *Alternaria alternata*, four seed samples were selected for further studies. The seed samples were collected from Jhansi (Sample No. 21 having 28.00% association), Mahoba (Sample No. 27 having 30.00%), Banda (Sample No. 30 having 32.00% association) and Hamirpur (Sample No. 24 having 28.0% association) (Table 8).

7.4. Association of mycoflora with deformed and shrunked chilli seeds

On the basis of naked eye observation, the seeds of four chilli samples were classified into two broad groups. One group contained bold apparently healthy seeds and second with shrunked and deformed seeds (Fig. 2). They did not include damaged seeds. By using Standard Blotter Method (ISTA, 1985), the two type of chilli seeds were analysed for the association of *Alternaria alternata* and other mycoflora and results are presented in Table 10.

Table 10. Association of mycoflora with bold apparently healthy seeds and deformed shrunked seeds of four seed samples of chilli as detected by Standard Blotter Method

Mycoflora	Seed samples/% mycoflora associated									
	Jhansi		Hamirpur		Mahoba		Banda		Average	
	A	B	A	B	A	B	A	B	A	B
<i>Alternaria alternata</i>	26	28	30	30	30	31	28	32	28.5	30.25
<i>Aspergillus flavus</i>	2	6	0	6	0	4	4	8	1.5	6.00
<i>Aspergillus niger</i>	2	2	0	4	3	2	2	2	1.7	2.50
<i>Aspergillus fumigatus</i>	0	2	1	2	1	2	2	2	1.0	2.00
<i>Curvularia lunata</i>	4	3	3	1	4	1	4	6	3.7	2.70
<i>Colletotrichum capsici</i>	12	18	8	6	6	8	2	2	7.0	8.50
<i>Cladosporium oxysporum</i>	2	2	0	4	2	4	3	4	1.7	3.50
<i>Fusarium oxysporum</i>	4	2	0	2	0	2	2	2	1.5	2.00
<i>Penicillium sp.</i>	2	1	2	0	2	2	4	2	2.5	1.20
<i>Rhizopus sp.</i>	1	0	0	1	2	2	2	2	1.2	1.20
Total frequency	55	64	43	56	50	58	52	62		
% germination	40	31	40	38	60	52	48	34		

A= Apparently healthy seeds

B= Shrunked seeds

In all, 10 fungi were recorded from the seeds. Apparently healthy and bold seeds had lesser number of fungi as compared to shrunked seeds. Maximum number of fungi (114) were recorded from the both sample collected from Banda.

Among apparently healthy and bold seeds of all the four seed samples, maximum 32.0% association of *Alternaria alternata* was recorded, while minimum 26.0% association was recorded. Average with healthy seeds as compared to 30.25% with shrunked rusty seeds. Association of other fungi in shrunked seeds was comparatively higher *Colletotrichum capsici* (8.5%), *Aspergillus flavus* (6.0%), *Curvularia lunata* (3.70%) and *Cladosporium oxysporum* (3.5%) (Table 10).

8. Efficacy of various methods in the detection of *Alternaria alternata* and other mycoflora associated with four selected chilli seed samples

Four methods were tested for the association of *Alternaria alternata* with the samples obtained from Jhansi (Sample No. 21.), Hamirpur (No. 24), Mahoba (No. 27) and Banda (No. 30). Two hundred seeds of each sample were tested. Observations on the associated fungi were made on the basis of their colony character, habit character under stereoscopic binocular microscope and finally by examining the slides under compound microscope.

8.1. Standard Blotter Method (ISTA, 1985)

The percentage of mycoflora associated with untreated and pretreated chilli seeds of four samples is given in Table 11.

The per cent incidence of association of fungi varied in pretreated and untreated seeds. Untreated seeds of chilli showed greater association of fungi and ranged from 47 to 52%, while pretreated seeds had a range of 26.0 to 55.0%.

In all, 15 fungi were recorded (Table 11). The association of *Alternaria alternata* was 28.0, 30.0, 30.0 and 30.0% in samples from Jhansi, Hamirpur, Mahoba, Banda respectively while it was 20.0, 24.0, 20.0, 30.0% after treatments of the seeds, respectively. Average association of *A. alternata* was 29.5%, while in pretreated seeds 25.5% association was observed (Table 11).

The germination of seeds ranged from 43.0 to 78.0%. Maximum seed germination (78%) was recorded in the sample from Mahoba after treatment.

Table 11. Association of mycoflora with four samples of chilli seeds as detected by Standard Blotter Method

Mycoflora	Seed samples/% mycoflora associated									
	Jhansi		Hamirpur		Mahoba		Banda		Average	
	U	P	U	P	U	P	U	P	U	P
<i>Alternaria alternata</i>	28	20	30	24	30	28	30	30	29.5	25.5
<i>Aspergillus flavus</i>	0	2	0	2	6	0	0	0	1.5	1.0
<i>Aspergillus niger</i>	2	0	2	1	0	0	0	1	1.0	0.5
<i>Aspergillus fumigatus</i>	1	0	2	0	2	0	0	5	1.2	1.2
<i>Botrytis cinerea</i>	3	0	2	1	2	0	0	4	1.7	1.2
<i>Chaetomium sp.</i>	0	0	2	2	0	2	4	4	1.5	2.0
<i>Curvularia lunata</i>	3	0	1	0	0	3	0	0	1.0	0.7
<i>Colletotrichum capsici</i>	16	2	6	2	8	5	0	0	7.0	2.2
<i>Cladosporium oxysporum</i>	0	0	2	1	4	6	0	2	1.5	2.2
<i>Drechslera tetramera</i>	4	2	0	2	2	0	2	0	2.0	1.0
<i>Fusarium oxysporum</i>	1	0	6	1	0	2	2	2	2.2	1.2
<i>Fusarium solani</i>	2	0	0	1	2	0	0	5	1.0	1.5
<i>Memnoniella sp.</i>	0	0	0	1	1	0	0	0	0.2	0.2
<i>Penicillium sp.</i>	1	0	0	2	0	0	4	0	1.5	0.5
<i>Rhizopus sp.</i>	0	0	0	2	0	1	1	2	0.2	1.2
Total frequency	52	26	53	42	57	47	47	55	-	-
% germination	43	48	48	54	70	78	54	64	-	-

U = Untreated seeds

P = Pretreated seeds

8.2. Agar-plate method (Modified Ulster Method-Muskett and Colhoun, 1948)

In this method potato-dextrose-agar (PDA) was used. Ten chilli seeds were plated on PDA in each petridish. Prior to plating the seeds were treated with 0.1% mercuric chloride for 30 seconds, followed by thorough washing with sterile water.

Average association of *Alternaria alternata* was 19.0% in the sample with 12.0, 18.0, 22.0, and 24.0% in the sample of Jhansi, Hamirpur, Mahoba and Banda, respectively (Table 12).

In all, eight fungi were found associated with the samples when tested by agar plate method. Other important fungi were *Colletotrichum capsici* (45.0%), *Curvularia lunata* (1.5%) and *Fusarium oxysporum* (1.5% average).

Germination of seeds was less on PSA medium. Only 52.0% could germinate in Jhansi sample and 46, 64 and 68% in Hamirpur, Mahoba, and Banda seed sample, respectively.

Table 12. Association of mycoflora with four samples of chilli seeds as detected by Standard Agar Plate Method

Mycoflora	Seed samples/% mycoflora associated				
	Jhansi	Hamirpur	Mahoba	Banda	Average
<i>Alternaria alternata</i>	12	18	22	24	19.0
<i>Aspergillus niger</i>	0	0	2	0	0.5
<i>Aspergillus flavus</i>	2	1	0	0	0.7
<i>Colletotrichum capsici</i>	12	6	0	0	4.5
<i>Curvularia lunata</i>	2	2	2	0	1.5
<i>Fusarium oxysporum</i>	1	3	0	2	1.5
<i>Fusarium solani</i>	0	0	0	2	0.5
<i>Penicillium sp.</i>	2	0	2	1	1.2
Total frequency	31	30	28	29	-
% germination	52	46	64	68	-

8.3. 2, 4-D Method (Neergaard, 1973)

2, 4-D was tried by Neergaard (1973, 1977) for the first time in the detection of mycoflora associated with seeds during routine seed health testing in Denmark. In the present investigation, the sodium salt of 2, 4-D

was used in two ways. In the first method the seeds were soaked in 2000 ppm solution of 2, 4-D for 15 minutes and thereafter plated on blotters wetted with sterile water. In the second method, the blotters were dipped in 2, 4-D solution and seeds were plated in Standard Blotter Method and observations on eighth day of incubation were recorded for the association of mycoflora. The data are presented in Table 13.

In this method, overall eight fungi were found associated with chilli seeds. The maximum association of *Alternaria alternata* was 26.0% when seeds were soaked in the salt, whereas it was 25.0% in case of blotter soaked in the solution of 2, 4-D. For the detection of *A. alternata* the blotter dip method was better than seed soak method in view of the seeds exhibiting higher average association 18.5% than 18.0% (Table 13), respectively.

The germination of seeds ranged from 52.0 to 79.0%. Non significant clear variation was observed as the influence on germination of both the methods were almost equal.

Table 13. Association of mycoflora with four samples of chilli seeds as detected by 2, 4-D Method

Mycoflora	Seed samples / % mycoflora associated									
	Jhansi		Hamirpur		Mahoba		Banda		Average	
	BD	SS	BD	SS	BD	SS	BD	SS	BD	SS
<i>Alternaria alternata</i>	18	12	13	12	18	26	25	22	18.5	18.0
<i>Aspergillus niger</i>	3	4	0	4	4	2	0	0	1.7	2.5
<i>Curvularia lunata</i>	0	4	2	2	0	0	0	0	0.5	1.5
<i>Colletotrichum capsici</i>	8	8	0	2	2	0	4	0	3.5	2.5
<i>Cladosporium oxysporum</i>	2	2	0	4	2	2	2	2	1.5	2.5
<i>Fusarium solani</i>	0	0	4	0	0	2	0	2	1.0	1.0
<i>Penicillium sp.</i>	2	1	0	2	0	2	0	0	0.5	1.2
<i>Rhizopus sp.</i>	1	0	0	1	0	3	0	3	0.2	1.7
Total frequency	34	31	19	27	26	37	31	29	-	-
% germination	62	52	71	68	74	79	70	69	-	-

BD = Blotter dipped technique SS = Seed soaking technique

8.4. Deep Freezing Blotter Method (Limonard, 1968)

In this method, chilli seeds were plated on moist blotters as in standard blotter method and were incubated for 24 hours, thereafter the plates were transferred to deep freeze having temperature 7°C in complete darkness for 24 hours. Then the plates were again placed in the growth chamber for five days. The observations were recorded on the association of mycoflora and results are presented in Table 14.

Data presented in Table 14 indicate that the association of *Alternaria alternata* ranged from 10.0 to 18.0%. The average association of the fungus was 14.5%. In this method, overall six fungi were found associated with seeds. Seeds from Jhansi exhibited 18.0% association, while 10.0, 14.0 and 16.0% was observed in the seed samples from Hamirpur, Mahoba and Banda, respectively.

In this method, germination of chilli seeds was nil due to very low temperature the seed embryo was dead. The death of embryo helped in better detection as the ungerminated seeds remained at the same place and level and are not disturbed from their place.

Table 14. Association of mycoflora with four samples of chilli seeds as detected by Deep Freeze Blotter Method

Mycoflora	Seed samples/% mycoflora associated				
	Jhansi	Hamirpur	Mahoba	Banda	Average
<i>Alternaria alternata</i>	18	10	14	16	14.5
<i>Aspergillus flavus</i>	0	0	1	0	0.2
<i>Curvularia lunata</i>	4	0	0	2	1.5
<i>Colletotrichum capsici</i>	4	2	0	2	2.0
<i>Fusarium oxysporum</i>	8	8	0	2	4.5
<i>Penicillium sp.</i>	2	1	0	0	0.7
Total frequency	36	21	15	22	-
% germination	0	0	0	0	-

9. Efficacy of modified methods in the detection of *Alternaria alternata* associated with chilli seeds

Four methods *i.e.* Standard Blotter Method, Agar-plate Method, 2, 4-D Method and Deep Freeze Blotter Method were tried for the detection of *Alternaria alternata* with chilli seeds. In further studies, these methods were tried with some modifications, with the expectation of higher counts of *A. alternata*.

9.1. Modification of Standard Blotter Method

In this method, the initial pH of water used for wetting circular pieces of blotters was changed with the help of 0.1 N HCl and 0.1 N NaOH and three levels of pH *i.e.* 6, 7 and 8 were used. Rest of the method was the same as in Standard Blotter Method. Observations on the association of mycoflora with chilli seeds were made on the eighth day.

Data obtained in Table 15 indicate that 12 fungi were found associated with seeds. The association of fungi varied with pH levels.

Maximum association of *Alternaria alternata* was 28.0% at pH 8 in the sample from Mahoba, while the range of association was 22.0 to 28.0%. Minimum association was in the sample (22.0%) from Banda at pH 8.

On the average basis, the maximum association was recorded at pH 6, where 26.6% chilli seeds exhibited the infection followed by 25.0% association at pH 7 and 8. Other important fungi observed were *Colletotrichum capsici* (ranged from 6 to 6.5% average infection), *Curvularia lunata* (2.5 to 4.0% average infection) and *Fusarium oxysporum* and *Fusarium solani* (1.5 to 2.5% infection).

The germination percentage of the seeds ranged from 50 to 70% at pH 7 germination was better.

Table 15: Influence of three pH levels of water used is standard Blotter method for the detection of *Alternaria alternata* associated with four seed samples of chilli

Mycoflora	Seed samples/% mycoflora associated at three pH levels														
	Jhansi			Hamirpur			Mahoba			Banda			Average		
	6	7	8	6	7	8	6	7	8	6	7	8	6	7	8
<i>Alternaria alternata</i>	25	25	25	28	24	24	26	27	28	28	24	22	26.6	25.0	24.7
<i>Aspergillus niger</i>	2	1	1	2	4	6	4	3	0	2	3	4	2.5	3.0	2.7
<i>Aspergillus flavus</i>	1	0	2	1	2	1	0	0	1	6	6	6	2.0	2.2	2.5
													5		
<i>Aspergillus fumigatus</i>	2	1	1	0	2	2	2	0	2	0	1	0	1.0	2.0	1.2
														5	
<i>Botrytis cinerea</i>	2	3	2	2	4	2	2	3	0	3	2	1	2.2	2.5	1.2
<i>Curvularia lunata</i>	5	3	1	3	2	6	2	2	2	6	4	1	4.0	2.7	2.5
<i>Colletotrichum capsici</i>	12	12	3	5	4	8	6	8	10	2	0	4	6.0	6.0	6.5
<i>Chaetomium sp.</i>	0	2	0	2	0	4	2	0	2	2	4	2	1.5	1.5	2.0
<i>Fusarium oxysporum</i>	2	4	4	4	0	0	2	0	1	0	2	2	2.0	1.5	1.7
<i>Fusarium solani</i>	0	0	2	2	2	2	4	4	2	4	2	0	2.5	2.0	1.5
<i>Penicillium sp.</i>	0	3	1	6	0	0	4	6	0	4	1	2	3.5	2.5	0.7
														0	
<i>Rhizopus sp.</i>	4	0	1	2	1	2	1	1	1	4	0	0	2.7	0.5	1.0
Total frequency	55	54	43	57	45	57	55	54	48	61	49	44	-	-	-
% germination	50	52	50	52	54	53	65	70	62	58	57	56	-	-	-

9.2. Modification of Agar-Plate Method

Agar plate method was modified by incorporating chilli seed extract in potato dextrose agar medium. Observations were recorded on seeds plated on PDA+Seed extract medium and plates were incubated for five days in growth chamber.

Data presented in Table 16 indicate that in all seven fungi were found associated with chilli seeds. In seed sample from Jhansi the association of *A. alternata* was 16.0% while 18.0, 28.0 and 22.0% association was recorded in the samples from Hamirpur, Mahoba and

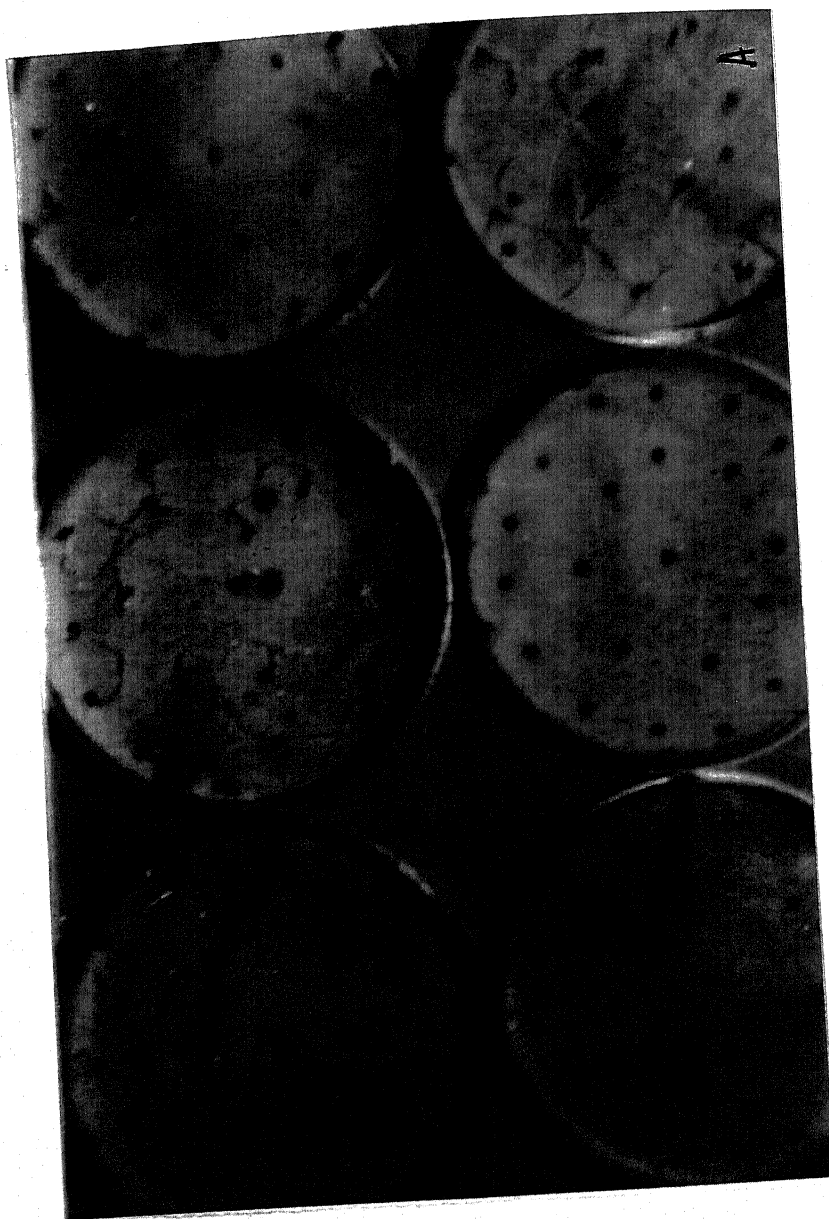


Fig.8. Effect of seed germination by standard Blotter Method.

Banda, respectively. An average of 21.0% association of the fungus was recorded.

Germination of chilli seeds was very less and it ranged from 18.0 to 30.0% (Table 16).

Table 16. Per cent association of mycoflora with chilli seeds detected by modified agar plate method on potato sucrose agar medium amended with chilli seed extract

Mycoflora	Seed samples/% mycoflora associated				
	Jhansi	Hamirpur	Mahoba	Banda	Average
<i>Alternaria alternata</i>	16	18	28	22	21.00
<i>Aspergillus niger</i>	1	2	2	2	1.75
<i>Aspergillus flavus</i>	4	0	0	2	1.5
<i>Botrytis cinerea</i>	1	0	2	2	1.25
<i>Curvularia lunata</i>	1	2	1	1	1.25
<i>Colletotrichum capsici</i>	4	3	0	0	1.75
<i>Fusarium oxysporum</i>	2	0	1	3	1.5
Total frequency	29	25	34	33	-
% germination	30	23	18	20	-

9.3. Modification of Deep Freeze Blotter Method

In this method, blotters were soaked in 2000 ppm solution of streptomycin sulphate. Extra solution of antibiotic was drained off and chilli seeds were plated on moist blotters as in Standard blotter method. Petridishes were transferred to low temperature for 24 hr, then re-incubated for five days in the growth chamber.

It is clear from the data presented in Table 17 that in blotter dip technique the average association of *A. alternata* was greater (18.0%) as compared to blotter dipped in sterile water (17.0%) in the modified deep freeze blotter method (Fig. 8). In this method, maximum counts of *A.*

alternata ranged from 14.0 to 20.0% being maximum in blotter dip method and samples from Jhansi, Mahoba and Banda. Streptomycin sulphate inhibited the bacterial contamination in petridishes. None of the seed germinated as they were exposed to freezing temperature due to which the embryo was killed.

Table 17. Efficacy of streptomycin sulphate in the detection of *Alternaria alternata* associated with chilli seeds as detected by modified deep freeze blotter Method

Mycoflora	Seed samples/% mycoflora associated									
	Jhansi		Hamirpur		Mahoba		Banda		Average	
	SP	UT	SP	UT	SP	UT	SP	UT	SP	UT
<i>Alternaria alternata</i>	20	14	16	17	18	17	18	20	18.0	17.0
<i>Aspergillus niger</i>	1	1	2	1	3	2	1	4	1.75	2.0
<i>Curvularia lunata</i>	3	3	2	3	3	5	1	0	2.25	2.25
<i>Colletotrichum capsici</i>	4	9	8	2	3	3	2	4	4.2	4.5
<i>Fusarium oxysporum</i>	8	10	7	4	1	4	6	7	5.5	5.0
<i>Alternaria solani</i>	0	1	4	3	8	4	1	6	3.25	3.5
<i>Penicillium sp.</i>	2	1	1	0	1	1	1	1	1.25	0.75
Total frequency	38	39	40	30	37	37	30	42	-	-
% germination	0	0	0	0	0	0	0	0	-	-

SP = Blotter dipped in streptopenicillin (2000 ppm)

UT = Blotter dipped in sterile water (untreated blotter)

10. Comparative efficacy of methods for the detection of *Alternaria alternata* associated with chill seeds

Comparative efficacy of different methods for the detection of *A. alternata* association with chill seeds was tried in 12 ways on four selected seeds sample obtained from Jhansi, Hamirpur, Mahoba and

Banda exhibiting the maximum natural infection of the fungus among 50 seed samples tested by Standard Blotter Method. The result of comparative efficacy of the methods are summarised in Table 18.

The association of *A. alternata* was maximum 32.00% in untreated seeds of chillies as tested by Standard Blotter Method (ISTA, 1985) while the minimum (14.00%) in agar plate method. On the average basis, the maximum (30.00%) association of the fungus was in untreated seeds of Standard Blotter Method and minimum (14.00%) in deep freeze blotter method. It was also interesting to note that the modified method of standard methods employed with the expectation of higher counts of the association of *A. alternata* were practically almost nonsignificant. No desired variation could be achieved (Table 18).

Table 18. Comparative efficacy of various methods in the detection of *Alternaria alternata* associated with chilli seeds

Method		% association of <i>Alternaria alternata</i>		
		Minimum	Maximum	Average
1.	Standard Blotter			
i)	Untreated seeds	28.0	32.0	30.0
ii)	Pretreated seeds	22.0	30.0	26.0
2.	Agar plate			
i)	PSA	14.0	23.0	18.5
3.	2, 4-D (2000 ppm)			
i)	Blotter dip	14.0	27.0	20.5
ii)	Seed soak	10.0	26.0	18.0
4.	Deep freeze blotter	12.0	18.0	15.0
5.	Modified Standard Blotter (pH level of water)			
i)	6	27.0	28.0	27.5
ii)	7	23.0	26.0	24.5
iii)	8	20.0	30.0	25.0
6.	Agar plate (PSA + Chilli seed extract)	18.0	28.0	23.0
7.	Modified Deep Freeze			
i)	Blotter dip in Streptopenicillin (2000 ppm)	16.0	19.0	17.5
ii)	Sterile water	12.0	20.0	16.0

11. Factors affecting the growth and sporulation of *Alternaria alternata*

Various factors influencing growth, sporulation and spore germination were studied. Effect of temperature, media and hydrogen ion concentration (pH) was studied on the colony characters of the fungus isolated from chilli fruits. Influence of exogenous supply of carbohydrate, chilli seed exudates, relative humidity, temperature was studied by cavity slide spore germination and results are presented.

11.1 Effect on growth

11.1.1 Effect of media

Growth of *A. alternata* was studied on ten synthetic and non-synthetic media. Colony was measured after 5 and 8 day incubation of inoculated plates and results are presented in Table 19.

It is clear from the data presented in table 19 that maximum colony diameter 86.0 mm was recorded in potato dextrose agar medium after 8 days incubation, followed by chilli seed extract agar and Richard's agar (82.0 mm). Among the 10 media, the minimum colony diameter of 51.5 mm was observed on soil extract agar medium.

In RMA, CMA, SEA and OMA the colony growth was almost submerged while in PDA, CSEA, CA, AHA, RA and MA the growth was fluffy.

The pigmentation appeared on the back of Petridishes also differed. Typical olive green black colour with concentric rings was observed on PDA and Czapeck's agar medium.

In CMA, RMA and OMA the brown pigmentation was recorded while in rest of the media the pigmentation ranged from light green to black. The sporulation also differed and ranged from 7 to 35 spores per microscopic

field being the maximum in PDA and least in OMA. The submerged colony growth had lesser number of spores (Table 19).

Table 19. Effect of ten synthetic and non-synthetic media on the growth and sporulation of *Alternaria alternata* isolated from chilli fruits

Medium	Colony diameter (mm)/day		Colony pigmentation	Colony type	No. of Spores per microscopic field
	5	8			
Potato dextrose agar	41.0	86.0	Olive green/black	Fluffy	35
Rice meal agar	35.8	62.5	Light brown	Submerged	21
Corn meal agar	49.5	73.0	Brown	Submerged	10
Soil extract agar	21.5	51.5	Light green	Submerged	7
Chilli seed extract agar	36.0	82.5	Light green	Fluffy	30
Oat meal agar	33.0	73.0	Light brown	Submerged	8
Czapek's agar	26.0	61.5	Olive green	Fluffy	26
Asthana and Hawker's agar	40.0	69.5	Light green	Fluffy (Partial)	22
Richard's agar	47.6	82.6	Green/black	Fluffy	29
Martin's agar	35.8	79.5	Light green	Fluffy (Partial)	14

11.1.2 Effect of temperature

Influence of four levels of temperature was determined on the growth of *A. alternata*. The inoculated petridishes were exposed to 20, 25, 30, and 35°C. The data are presented in table 20.

It is clear from the data presented in table 20 that the fungus grew at 30°C. The colony diameter was maximum 91.0 mm at 30°C, followed by 85.0 mm at 25°C and least 60.5mm at 35°C after 8 days of incubation.

The colony of *A. alternata* was fluffy in all the treatments and

pigmentation was light green to olive green black with clear concentric zones. Maximum spores (34 per microscopic field) were recorded at 30°C while minimum (25) at 35°C.

Table 20. Effect of four levels of temperature on the growth and sporulation of *Alternaria alternata* from chilli fruits

Temperature °C	Colony diameter	(mm)/ days	Colony pigmentation	Colony type	No. of spores per microscopic field
	5 Days	8 Days			
20	32.5	63.0	Olive green	Fluffy	26
25	46.5	85.0	Olive green/black	Fluffy	27
30	42.0	91.0	Light green	Fluffy	34
35	31.6	60.5	Olive green	Fluffy	25

11.1.3 Effect of Hydrogen ion Concentration (pH)

Eight levels were used to determine the effect of different pH on the growth and spores number of *A. alternata* and result are presented in table 21.

It is clear from the data present in table 21 that maximum diameter 90.5 mm was recorded at pH 6.5, followed by 85.0 mm at pH 6 and 82.6 mm at pH 7.00. The minimum diameter 59.5 mm was recorded at pH 4.5.

At all the pH levels, the colony was fluffy type and pigmentation ranged from light brown to typical green black with concentric rings. The number of spores ranged from 12 to 29 per microscopic field.

Maximum (29) spores were recorded at pH 6.5. It appears from the data that fungus grew well at all the pH levels, except pH 4.5 where the colony diameter was only 59.5 mm after 8 days incubation, whereas at pH 8.0

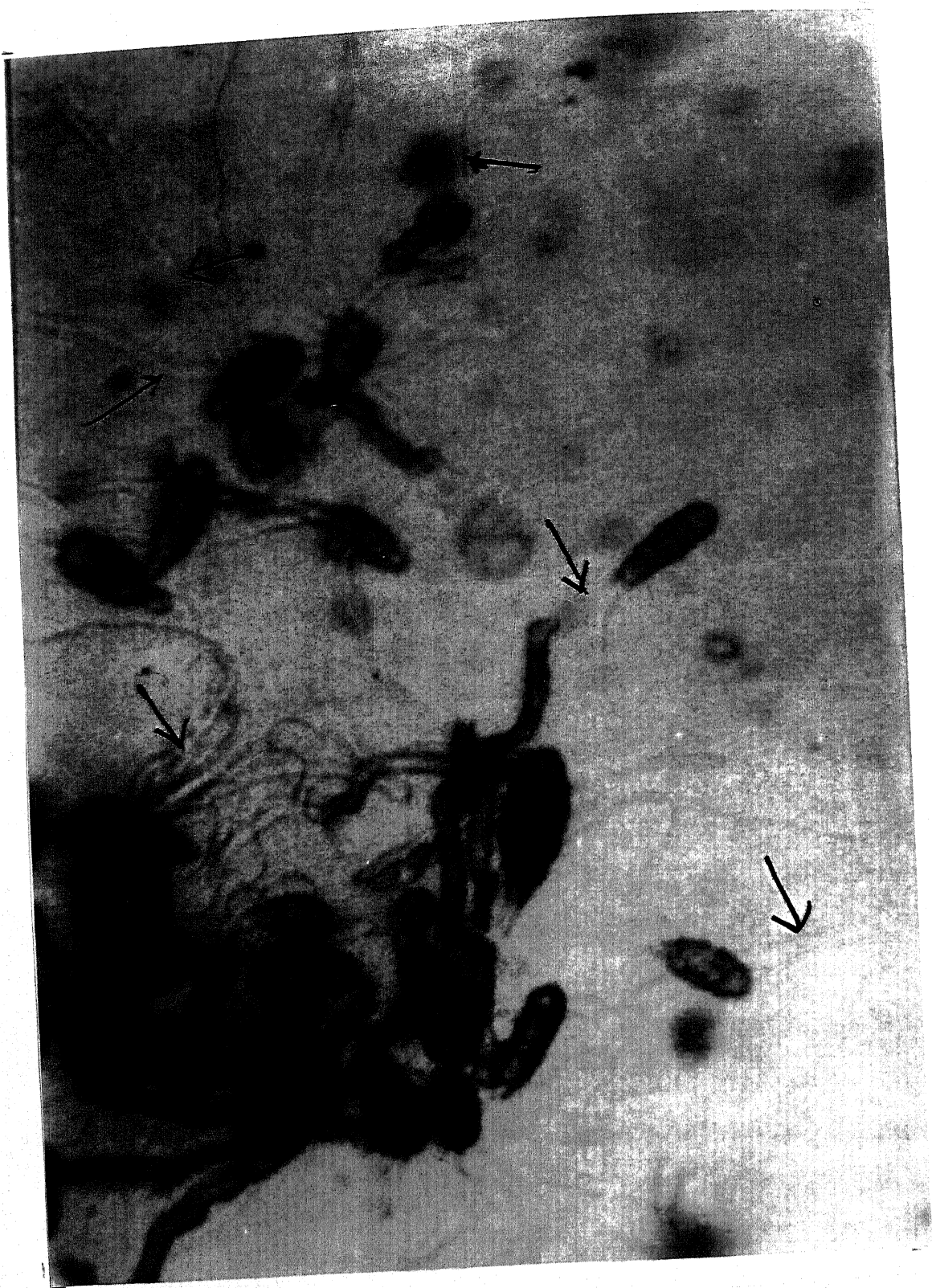


Fig.9. Unipolar, Bipolar and septal germination of *A. alternata*.

the colony diameter was 79.0 mm but the number of spores was quite less (12) as compare to pH 4.5 (21) (Table 21).

Table 21. Effect of eight pH levels of media on the growth of *Alternaria alternata* isolated from chilli fruits

pH levels	Colony Diameter (mm)/days		Colony pigmentation	Colony type	No. of spores per microscopic field
	5	8			
4.5	22.5	59.5	Light brown	Fluffy	21
5.0	38.0	71.6	Light brown	Fluffy	23
5.5	35.4	75.0	Brown black	Fluffy	26
6.0	42.0	85.0	Brown black	Fluffy	27
6.5	62.0	90.5	Green black	Fluffy	29
7.0	43.6	82.6	Green black	Fluffy	28
7.5	31.0	79.0	Light black	Fluffy	27
8.0	30.5	79.0	Light black	Fluffy	12

11.2. Effect on spore germination

11.2.1. Effect on temperature

Influence of four levels of temperature was observed on the spore germination of *Alternaria alternata* and the result are presented in Table 22.

The conidial suspension on 1 ml having about 14 conidia were placed in the cavity chamber. The cavity slides were placed in the moist chamber. The data presented in Table 22 indicate that conidia of *A. alternat* germinated within 4.30 hours at 22°C, 5 hours at 25°C and 27°C and 5.30 hours at 30°C while it took about 6.30 hours at 15°C Unpolar, bipolar and septal germination was recorded (Fig. 9).

Table 22. Effect of four levels of temperature on spore germination of *Alternaria alternata* isolated from chilli fruits

Temperature (°C)	% spore germination/time (hr)									
	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5
15	0	12.0	17.5	33.6	40.8	51.0	59.0	73.0	59.	100
22	14.0	26.0	45.5	61.5	82.0	99	-	-	-	-
25	10.7	21.5	41.0	52.0	69.6	88.0	98	-	-	-
27	0	18.6	21.6	38.0	69.0	81.0	100	-	-	-
30	0	16.5	28.6	41.0	64.0	71.0	86.0	100	-	-

11.2.2. Effect of relative humidity

Influence of four levels of humidity was recorded on the spore germination of *A. alternata* and the result are presented in Table 23. The spores were kept dry and placed at different relative humidities.

The data presented in Table 23 indicate that at 90% RH, the germination started within 6 hours and completed in 18 hours. At 75% the germination started within 12 hours and only 61% germination was recorded within 18 hours. At 25% no germination was observed after 18 hours.

Table 23. Effect of four levels of relative humidity on spore germination of *Alternaria alternata* isolated from chilli fruits

Relative Humidity (%)	% spore germination/hours						
	6	8	10	12	14	16	18
90	11.0	15.0	22.0	44.0	75.0	88.0	99
75	0	0	0	13.0	24.0	43.0	61.0
50	0	0	0	0	4.5	22.0	31.0
25	0	0	0	0	0	0	0

11.2.3. Effect of exogenous supply of carbohydrates

Influence of exogenous supply of carbohydrates as sucrose was studied on the spore germination of *A. alternata* and result are presented in Table 24.

Five concentration 0.01, 0.25, 0.50, 1.00 and 1.50% of sucrose were used and distilled water (Without sucrose) served as control for the preparation of spore suspension. It is clear from the Table 24 that at 0.01 and 0.25% concentration the germination was completed within 3 hours while it took 5 hours in control and 1.0% concentration, while after 5 hours only 68.0% germination was recorded at 1.5% concentration. In control, the germination of *A. alternata* spores started after 3 hours while at low concentration started within one hour indicating the influence of exogenous supply of sucrose as acceleration in spore germination (Table 24).

Table 24. Effect of five levels of sucrose concentration on the spore germination of *Alternaria alternata* at 25°C

Sucrose concentration (%)	% germination/time (hr)				
	1	2	3	4	5
1.5	0	26	41	58	68
1.0	0	48	73	89	100
0.5	30	64	88	98	-
0.25	38	68	98	-	-
-0.01	36	76	100	-	-
0.00	0	0	41.0	68	98

11.2.4. Effect of seed-exudate

Influence of seed exudate on the spore germination of *A. alternata* was observed and the data are recorded in Table 25.

It is clear from the data that in control the spore germination was

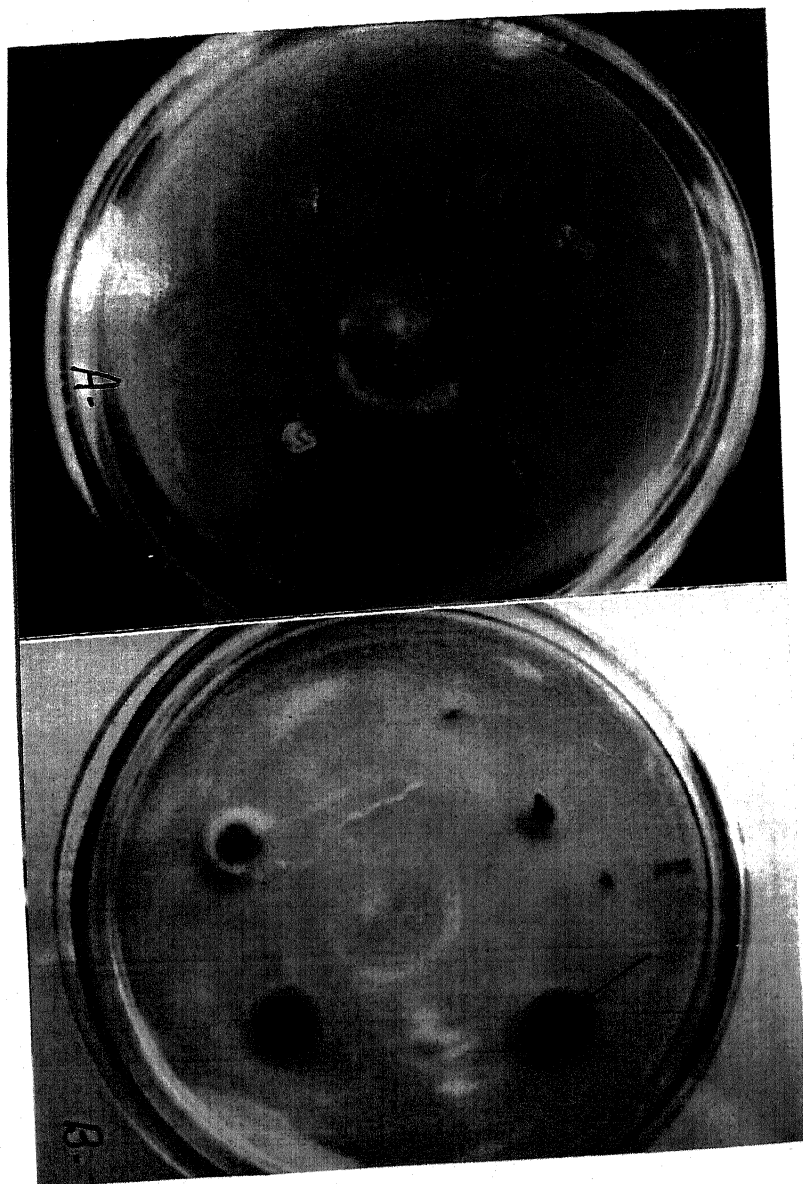


Fig.10. Effect of *A. alternata* transmitted from seed to plant.

96.0%. It is observed that the seed exudation had inhibitory effect on the spore germination of *A. alternata* isolated from chilli fruits (Table 25).

Table 25. Effect of seed exudates on germination of conidia of *Alternaria alternata*

Treatment	% germination after five hours
Control	96
Seed exudate	72

12. Transmission of *Alternaria alternata*

Transmission of the fungus was observed from seed to plant and plant to seed. The fungus was found associated with seeds, externally as well as internally, with leaves, twigs, stem and fruits. The infected parts which remain in the field serve as the source of infection.

12.1. Seed to plant transmission

Seed to plant transmission of *A. alternata* was studied and confirmed in the following ways and the result are presented in Table 26.

12.1.1 Seed to plant transmission and role of seed-born

A. alternata was studied by placing a seed (of sample No. 27) on solidified one percent water agar in test tube. Seed sample obtained from Pandurna having maximum natural infection upto 31.0% were used. It is clear from the data presented in Table 26 that after 12 days incubation only 56.0% seed germinated. Rest 44.0 percent seeds did not germinated, out of which 29.0% showed the infection of *A. alternata*. After 20 days, 8.00% mortality of the seedlings was recorded due to the same fungus. The movement of the fungus from seed to seedlings was recorded. The infected seed and seedlings yielded the growth of *A. alternata* on PDA (Fig. 10).

Table 26. Role of seed-borne *Alternaria alternata* in causing disease on chilli seedlings in the test tube seedling symptom method and in pots

Place	% germination	% pre-emergence mortality *		% Post emergence mortality **	
		Total	Due to <i>A. alternata</i>	Total	Due to <i>A. alternata</i>
Test tube with 1% water agar	56	44	29	12	8
Plastic pots with sterile sand/soil	43	57	27	10	9

* 12 days

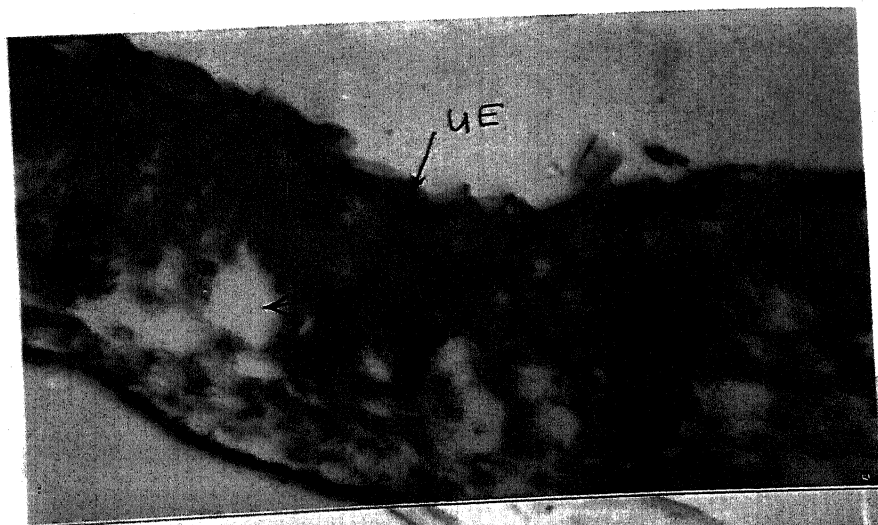
** 20 days

12.1.2. Naturally infected seeds obtained from Mahoba were shown in plastic pots containing sterile sand and observation on the disease incidence were recoded. Only 43.0% seeds germinated and 57.0% seed did not germinate. Upon isolation it was observed that 27.0% infection of *A. alternata* was confirmed. Post emergence mortality due to the fungus was observed in 10% seedlings (Table 26). Seed to seedling movement of the fungus was confirmed.

12.1.3. Site of infection of *A. alternata* was found out by component plating but exact components of chilli seeds could not be detected due to very fragile nature of seeds. However, the fungus could be observed externally as well as internally seed-borne. Observation under compound microscope on the cross section of the seeds with sharp razor blade revealed the presence of fungal remnants. In the distorted portion of the seed, a big cavity was formed and fungal mycelium was recorded.

12.1.4. Histopathological studies

To study the host parasite relationship, sections through healthy and diseased portion of leaves and stem of chilli were cut and following observation were made:



A



B



C

Fig.11. i) Effect of *A. alternata* in chilli leaf.

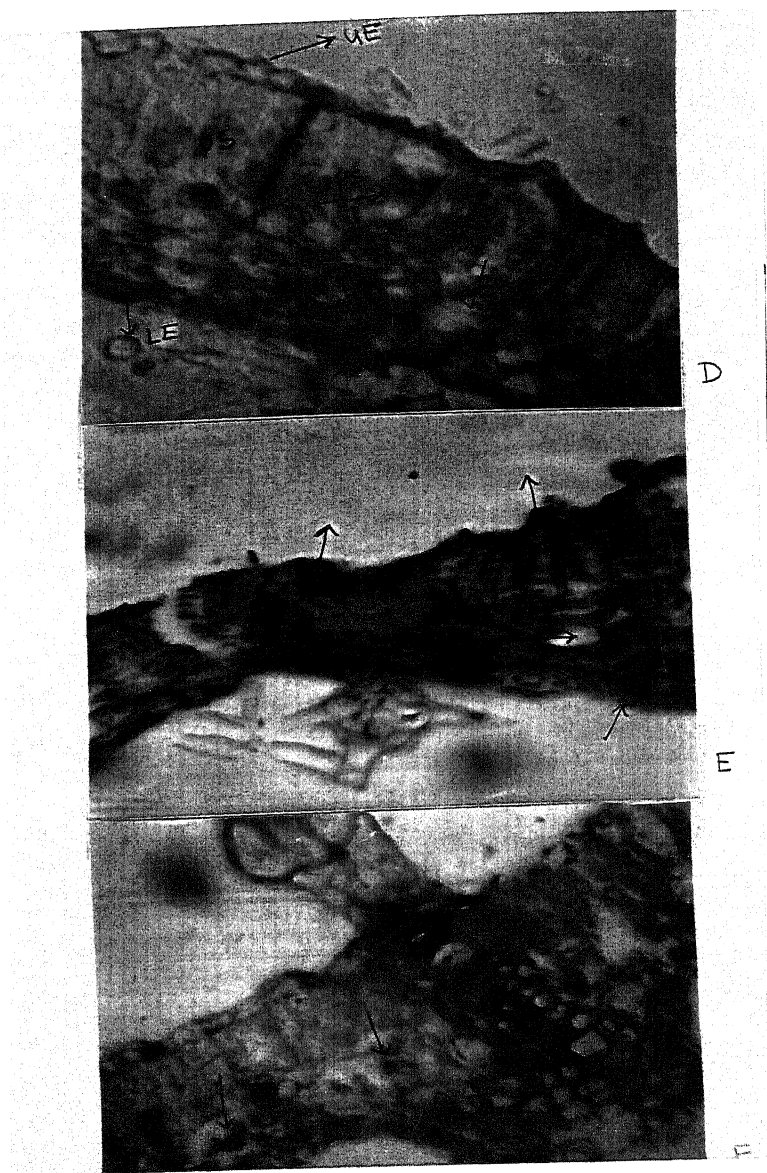


Fig. 11 ii) Effect of *A. alternata* on blocking of leaf V. Bundles.

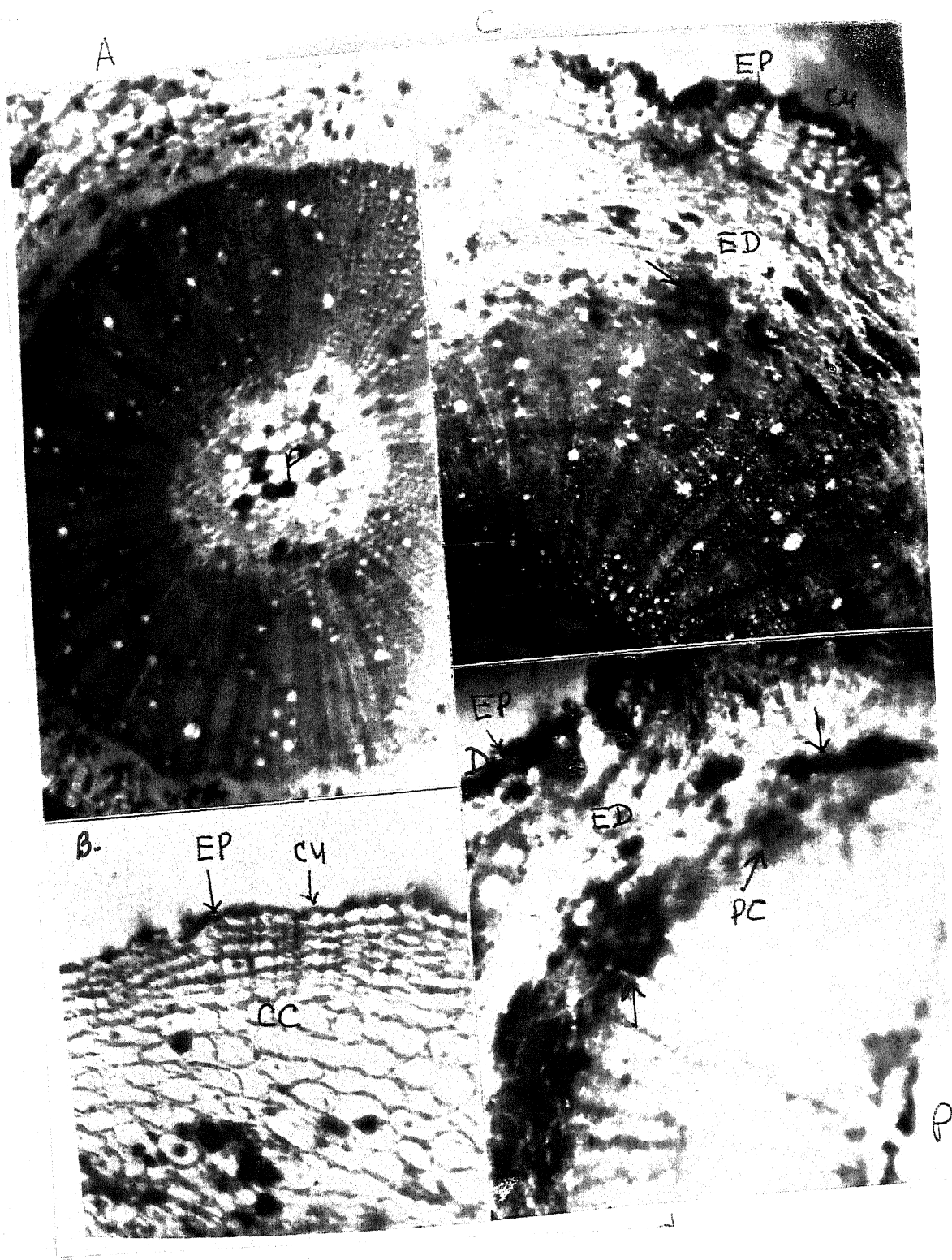


Fig.12. Effect of *A. alternata* on histological stem

12.1.4.1. Histology of infected leaves

Transverse section of lesions, cut by rotary microtom showed that infected cells became brown and collapsed. The fungus had colonised epidermis, pallisade and mesophyll tissue. the cell wall lost its shape as most of them collapsed.

In erlier stage myelium growth was disintegration seed but later, mycelial growth was noted (Fig. 11(i)). With the advancement of infection there was disintegration of phloem, however, the xylem was compratively less affected, although the fungal hyphae were seen in the xylem vessals. Small conidiophores were seen on the disintegrated and necrotic leaf lesions (Fig. 11(ii)). In the completely infected leaves the tissues could not be identified and separated. Due to infection, sevrsl cavities in the infected leaves were observed. Fungal growth was observed on lower as well as upper epidermis. Fungal hyphe was noted in the intercellular cavities. The pallisade cells and parenchymatous cells were seen disintegrating leading to the development of cavity.

12.1.1.2. Histology of infected stem

The transverse section of healthy stem of chilli exhibits epidermis with cuticle, cortical cells, endodermis, pericycle, phoem and xylem without the presence of the mycelium, while D shows the development of fungul infection in epidermal, subepidermal region only. Mycelial growth was not recorded in pith cell. The epidermal cells were deformed and disintegrated (Fig. 12).

12.2. Plant to seed transmission

12.2.1 Fruit inoculation technique

In this method, chilli fruits at three stages of growth, green, green-yellow and yello-red were incubated with 3 ml spore suspension of *A. alternata* through a hypodermic syringe (Needle No. 22), seeds harvested

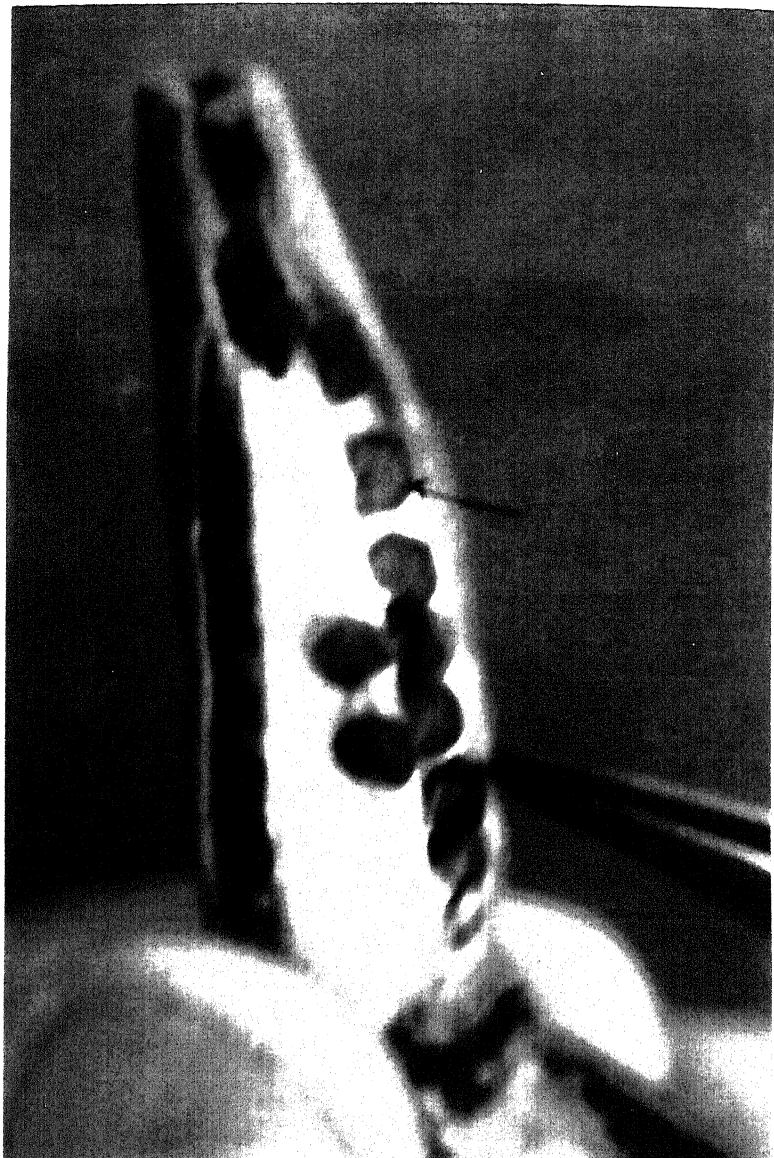


Fig.13. Effect of *A. alternata* transmitted plant to seed.

from inoculated fruit were tested for the association of the fungus and results are presented in table 27.

After harvesting, fruit inoculated at yellow-red stage exhibited 72% infection, green-yellow stage 67% and green stage 40%. In yellow-red stage infection, seed formation was not normal. Seeds were somewhat shrivelled and deformed as compared to green ones. When the yellow-red fruit were cut open, brownish black infected seeds were observed. Seeds harvested from artificially inoculated fruit at yellow-red, green-yellow and green were covered with fungal mycelial growth below the pericarp, the diseased area. The components of artificially inoculated fruits were plated on PDA. The pericarp, septum, placenta, seed and fruit stalk adjoining the calyx were plated. The fungal association was observed in pericarp, septum, seed and placenta but the adjoining portion of calyx did not show the infection (Table 27) (Fig. 13).

Table 27. Association of *Alternaria alternata* with fruit components

Fruit stage	Fruit component/Fungal colonization					% infection
	Pericarp	Seed	Septum	Placenta	Calyx	
Yellow-red	+	+	+	+	-	72.0%
Green-yellow	+	+	+	+	-	67.0%
Green	+	+	+	+	-	40.0%

(+) = Infection established

(-) = Infection not established

12.2.2. Naturally infected chilli fruits, twig, stem and leaves collected from field and were examined for the association of *A. alternata*. The fungus was found associated with all the plant parts. Each of the plant part yielded the fungus on PDA medium.

12.2.3. Infected fruits, twigs and seeds were collected and checked for the association of *A. alternata*. They were stored in paper envelopes at room temperature. After isolation on PDA, the viability of the cultuer was confirmed and the data are presented in the Table 28.

Table 28. Survival of *Alternaria alternata* associated with plant parts stored in paper envelops and field soil

Time interval (days)	% Viability of the fungus expressed in terms of growth on PDA			
	In Paper envelops			In sterile field soil in pots
	Twing	Fruit	Seed	Plant debris
30	+ (80)	+ (80)	+ (90)	+ (70)
60	+ (80)	+ (80)	+ (90)	+ (51)
90	+ (45)	+ (50)	+ (83)	+ (33)
120	+ (17)	+ (41)	+ (78)	+ (10)
150	- (0)	+ (15)	+ (40)	- (0)
180	-	- (0)	+ (25)	- (0)
210	-	-	+ (10)	- (0)

Survival of the fungus was analysed, it is clear from the data that fungus was viable in twigs upto 120 days, fruits 150 days and seeds even upto 210 days when kept in paper envelops. It was checked at 30 day interval by plating on PDA (Table 28). Initially the viabiality was upto 90 to 80% in twigs, fruits and seeds, and finally it was 10%, indicating the sufficient survival of fungus.

12.2.4. The infected parts were cut into small pieces, mixed and filled with sterile soil in pots. At monthly interval small bits were taken, washed and plated on PDA and the viability was confirmed.

The data presented in Table 28 indicates that isolation could be made upto only 120days indicating the survival upto only 4 months.

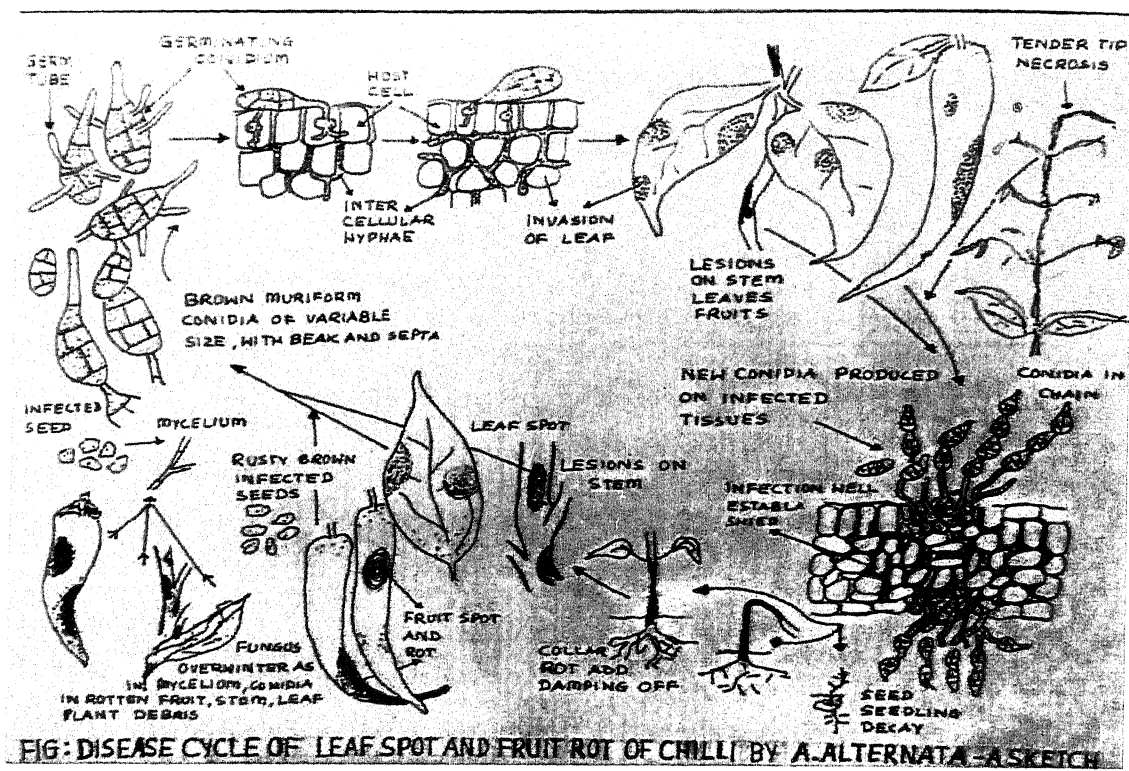


Fig.14. Showing the life cycle of *A. alternata*.

Initially the viability was only 70% and after 120 days only 10% viability was noticed.

12.2.5. Transmission of *A. alternata* from seed to plant and plant to seed was studied. An attempt was made to study the disease cycle of the leaf spot and fruit rot and based on the observation made, a digramatic sketch of the disease is prepared and presented (Fig. 14).

13. Production of toxic metabolites by *A. alternata*

13.1. Influence on seed germination

Culture filtrate of *Alternaria alternata* was prepared by growing it on Richard's medium in Erlenmeyer flask. Seed with out natural infection of the fungus collected from Kurvi were soaked in filtrate and the influence of culture filtrate on seed germination was observed. The data are presented in Table 29.

The data presented in Table 29 indicate that sterile water soaked seeds shows greater germination as compared to the seeds soaked in culture filtrate of 5, 10, 15, 25 and 30 days age. Maximum per cent reduction in seed germination was 70.24 percent when the seeds were soaked in culture filtrate from 15 day old culture. in sterile water seeds exhibited 84.0% germination as compare to only 25.0% in treated ones. After 15 days, culture filtrate treatment resulted in increase in germination percentage. The age of culture filtrate had clear cut influence (Plate 17). The germination of seeds ranged from 25.0 to 59.0% when treated with the filtrate of 5 to 30 days age as compared to 86.0% in sterile water. Minimum reduction seed germination was 31.40% when the seeds were treated with culture filtrate of 5 day old culture. After critical observation on seed germination, it was recorded at first (upto 10 days) the germination decreased thereafter (20 dayonword) it increased gradually indicating the maximum activity at 15 days old culture filtrate.

Table 29. Influence of culture filtrate of *Alternaria alternata* on germination of chilli seeds.

Age of culture from which culture filtrate obtained (days)	% seed germination		% reduction in germination
	Treated with culture filtrate	Treated with sterile water	
5	59	86	31.40
10	43	86	49.41
15	25	86	70.24
20	32	86	62.79
25	39	86	53.57
30	42	86	51.16

13.2. Influence of various dilutions on seed germination and shoot-root length

In the preliminary studies it was observed that the culture filtrate obtained from 15 day old culture was the most effective, hence the influence of various dilutions of this filtrate was tested and the results are presented in Table 30.

A general decrease in seed germination was confirmed when the seeds were treated with different dilutions of culture filtrate as compared to control and distilled water treatment. In crude culture filtrate the germination was only 20.0% as compared to 84.0' and 86.0%in filtrate obtained from uninoculated medium and distilled water, respectively (Table 30). Similarly as the crude culture filtrate, which considered as 100%, was diluted, the increased germination was observed, in 1:1 strength germination was 41.0%, 1:2 had 52.0 percent, 1:3 had 58.0%, 1:4 had 62.0% and 1:5 had 72.0%, indicating the ineffectivity of the metabolites.

Table 30. Influence of culture filtrate of *Alternaria alternata* on seed germination, root and shoot length

Treatment	% germination on blotters soaked 15 days	Root length (mm)	Shoot length (mm)
Distilled water (DW)	86.0	65.0	34.0
Culture filtrate (CF uninoculated)	84.0	62.0	34.0
Crude Culture Filtrate (CF)	20.0	3.0	2.0
CF : DW (1 : 1)	41.0	3.0	1.0
CF : DW (1 : 2)	52.0	6.0	0.0
CF : DW (1 : 3)	58.0	5.0	1.0
CF : DW (1 : 4)	62.0	12.0	3.0
CF : DW (1 : 5)	72.0	14.0	2.0

The root and shoot length also had adverse effect intreated and untreated seeds. In the treated seeds lesser lenght or greater inhibition of the length was recorded (Table 30). In crude culture filtrate the root length was only 3.0mm and shoot length was 2.0mm as compared 65.0 and 34.0 mm in distilled water and 62.0 and 34.0 mm in uninoculated filtrate. The vast difference in the length indicated the influence of the metabolites, however, it was intersting to note taht as the dilution increased the length was also somewhat increased. Root length was coparatively greater than shoot length.

13.3. Determition of absorpion time

The pretreated seeds of Puse Jalwa were soaked in the culture filtrate for 1, 2, 3, 6, 9 and 12 hours and the influence of soaking period was determined on the germination of seeds. The result are presented in Table 31. It is clear from the data that the increased. The seed soaked

with Richard's medium had almost similar germination and ranged from 63.0 to 73.0% while in case of seed soaked in culture filtrate for 1, 2 and 3 hour showed 18.0, 24.0 and 26.0% germination, respectively. seeds soaked in the filtrate more than 3 hours did not germinate, indicating the harmful nature of the metabolites (Table 31).

Table 31. Influence of soaking time of chilli seeds on their germination when treated with culture filtrate of *Alternaria alternata*

Soaking time (hr)	% germination	
	Toxin	Control
1	18.0	63.0
2	24.0	63.0
3	26.0	68.0
6	0.0	68.5
9	0.0	73.0
12	0.0	73.0

13.4. Influence of temperature on the activity of toxic metabolites produced by *A. alternata*

The data presented in Table 32 clearly indicate that when the culture filtrate was heated upto 50°C, it exhibited comparatively lesser seed germination as compared to the distilled water treated seeds. The germination percentage of 30, 40 and 50°C was at par with control where the 1:1 culture filtrate was used. Toxin heated upto 60°C or above had germination percentage at par with the germination in distilled water (Table 32).

Table 32. Influence of various temperatures on the activity of toxic metabolite of *Alternaria alternata* on germination of chilli seeds

Temperature ($^{\circ}\text{C}$)	% germination
Distilled water (DW)	87.0
30 $^{\circ}\text{C}$	35.0
40 $^{\circ}\text{C}$	31.0
50 $^{\circ}\text{C}$	30.0
60 $^{\circ}\text{C}$	80.0
70 $^{\circ}\text{C}$	80.0
80 $^{\circ}\text{C}$	83.0
90 $^{\circ}\text{C}$	83.0
Control (1:1) (CF : DW)	37.0

CF = Crude filtrate

DW = Distilled water

14. Factors affecting disease development

14.1. Under laboratory conditions

14.1.1. Influence of temperature

Fruits of Pusa Jwala and Jawahar Mirch of almost same age, size and source were brought and inoculated with pin prick method. The development of rot incited by *A. alternata* was recorded on 100 fruits after 10 and 15 days. The data are presented in Table 33.

It is clear from the data (Table 33) that the maximum (55%) increase in the rot was observed in Pusa Jwala at 25 $^{\circ}\text{C}$, followed by 30 $^{\circ}\text{C}$ (46.0%) and 35 $^{\circ}\text{C}$ (46.0%). In Jawahar Mirch-218 maximum 49% increase was recorded at 25 $^{\circ}\text{C}$, followed by 30 $^{\circ}\text{C}$ (45.0%) and 33.0% at 35 $^{\circ}\text{C}$. In both the varieties at lower temperature (10 $^{\circ}\text{C}$ and 15 $^{\circ}\text{C}$) disease

development was comparatively nil indicating the less activity of the fungus. There was no rotting at 10°C in both the varieties.

Table 33. Influence of different temperatures on the development of rot in chilli fruits of two varieties

Temperature °C	% fruit rot *				% Increase in fruit rot	
	Pusa Jwala		Jawahar Mirch 218		Pusa Jwala	Jawahar Mirch 218
	10 day	15 day	10 day	15 day		
10	0	0	0	0	0	0
15	4	10	2	13	4	10
20	16	33	20	49	17	29
25	33	87	42	91	54	49
30	28	73	43	85	45	42
35	11	57	18	51	46	33

*Pin pricked fruits

14.1.2 Influence of humidity levels on fruit rot

Fruits of Pusa Jwala and Jawahar Mirch 218 were brought and inoculated with *A. alternata* culture by pin prick method. These fruits were kept in desiccators maintained with different relative humidity levels. It is clear from the data presented in Table -34 that in all the humidity levels fruit rot developed after inoculation and ranged from 29.0 to 88.0% in both the varieties.

In Pusa Jwala maximum (48.0%) increase was noticed at 100% humidity level being 39.0% after 10 days and 87.0% after 15 days, where as minimum fruit rot (52.0%) was at 50% relative humidity after 15 days. In Jawahar Mirch 218 maximum fruit rot (81.0%) was recorded at 80%

relative humidity, followed by 80.0% at 90 and 100% relative humidity, while the maximum increase was 40.0% at 90% relative humidity.

It is also evident from the data that rotting was slower between 0 and 10 days of incubation as compared to 10 and 20 days of incubation (Table 34). Response of both the varieties also differed at different humidity levels.

Table 34. Influence of various levels of relative humidity on the development of rot in chilli fruits

Humidity level (%)	% fruit rot *				Increase in fruit rot	
	Pusa Jwala		Jawahar Mirch 218		Pusa Jwala	Jawahar Mirch 218
	10day	15day	10day	15day		
50	33	52	33	64	19	31
60	32	68	29	63	36	34
70	36	71	42	60	35	18
80	34	74	44	81	40	37
90	40	88	41	80	48	40
100	39	87	41	80	48	37

* Pin pricked fruits

14.1.3. Influence of age of chilli fruit

The effect of different age of chilli fruits on the development of rot after inoculation with *A. alternata* was determined and the results are presented in Table 35.

Table 35: Effect of different age on development of fruit rot of chilli after inoculating with *A. alternata*.

S.N	Stage	Per cent infection			
		Pandurna	Pusa Jwala	Jawahar Mirch 218	C-1 Pant
1.	Green	6.4	10.0	5.0	7.3
2.	Frish red turning	60.0	70.0	83.0	62
3.	Ful red ripe	7.0	9.0	6.0	5.5

Fruits of complete age i.e. full ripe were choosen and the turning stage was the criteria between the two ages. Observations after 15 days of inoculation indicated that as the age increased the rotting also increased; however, at red-ripe stage the infection rate was comparatively less.

Minimum infection (9.0%) was recorded at full red ripe stage in Pusa Jwala although at complete green stage also it was not susceptible where only 10.0% infection was recorded. The infection rate varied between complete green and complete red stage. On critical examination of data it is observed that maximum fruit rot (70.0%) was noticed when the fruits were at turning stage having yellow to red (II) skin colour. At this stage, red colour was dominated over yellow in the fruits. On the other hand, in Jawahar Mirch 218, the yellow red stage was also susceptible and maximum 83.0% fruit rot was recorded; however, in the yellow red (I) stage the yellow pigmentation was dominated. In Jawahar Mirch-218, minimum (5.0%) disease was recorded at green stage, followed by red ripe stage where only 6.0% fruit rot was recorded (Table 35).

It is evident from the data presented that yellow red stage was most susceptible as compared to green yellow. Similarly green stage was less susceptible as compared to red stage. In other words, young fruits were less susceptible.

15. Influence of fungal infection on the quality character of chilli fruits

Effect of infection of *Alternaria alternata* on ascorbic acid content, carotene and capsaicin was determined on the naturally infected fruits collected from Goara farm Banda. The results are presented in Table 36, 37 and 38.

15.1 Influence on ascorbic acid content

Vitamin C or ascorbic acid content is a major component of chilli. Its nutritive value is well known and established. Adverse effect of *A. alternata* infection on the Ascorbic acid content was observed and the results are presented in Table 36

Table 36. Influence of *Alternaria alternata* infection on the ascorbic acid content of four varieties of chilli fruits at three developmental stages

Stage		Ascorbic acid (mg 100g)			
		Pandurna	Pusa Jwala	Jawahar Mirch 218	C-1 Pant
1.	Green stage	113.78	135.90	207.66	250.10
2.	Fresh red turning	116.60	292.33	228.90	302.00
	Diseased	74.10	229.00	175.60	175.60
	% reduction	36.45	21.66	23.29	41.85
3.	Fresh red ripe	162.20	156.48	223.50	260.80
	Diseased	119.50	139.60	185.50	181.00
	% reduction	26.33	10.79	17.00	30.60

Ascorbic acid content was determined in green chillies, fresh red turning chillies and fresh red ripe chillies. It is clear from the data presented in Table 36 that ascorbic acid content decreased at all the three stages in all the four commonly used varieties.

In fresh red turning stage, maximum reduction of 41.85% was recorded in C-1 Pant, followed by Pandurna (36.45%), Jawahar Mirch 218 (23.29%) and Pusa Jwala (21.66%). Maximum ascorbic acid content was in C-1 Pant (302.00mg/100g), followed by Pusa Jwala (292.33), Jawahar Mirch 218 (228.90) and Pandurna (116.60mg/100g). At fresh red ripe stage, maximum reduction was 30.60% in C-1 Pant, followed by 26.33% (Pandurna), 17.00% (Jawahar Mirch 218) and 10.79% (Pusa Jwala).

As the fruit ripened the ascorbic acid content lowered down but remained higher as compared to green ones (Table 36).

15.2. Influence on carotene content

Effect on carotene content was determined in healthy and diseased fruits of chilli varieties and the results are presented in Table 37.

Table 37. Influence of *Alternaria alternata* infection on carotene content of four Varieties of chilli

Variety	Caroten content (mg/100g)		
	Healthy	Diseased	% reduction
Pandurna	0.178	0.132	25.84
Pusa Jwala	0.183	0.083	53.89
C-1 Pant	0.165	0.101	38.79
Jawahar Mirch 218	0.180	0.101	44.51

It is evident from the data that carotene content in healthy fruits ranged from 0.165mg/100g to 0.183 while in diseased from 0.083 to 0.132mg/100g (Table 37).

Maximum reduction in Pusa Jwala (53.89%) was recorded where 0.183mg was in healthy as compared to 0.083mg in diseased, followed by 44.51% in Jawahar Mirch 218, 38.79% in C-1 Pant and 25.84% in Pandurna.

15.3. Influence on capsaicin content

The pungency of chillies is due to an alkaloid-capsaicin ($C_{18}H_{27}NO_3$). The red colour in fruits at the ripening stage is due to the pigment-capsanthin. This alkaloid has been reported to be in all parts except seeds. It is present in septum also. The effect of fungal infection on the capsaicin content was determined and results are presented in Table 38.

Maximum reduction (49.65%) was noted in Pusa Jwala (425.0 as compared to 214.0 mg/100g in healthy and diseased, respectively), while minimum in Pandurna (21.57%). The capsaicin content reduction ranged from 21.57 to 49.65%.

Table 38. Influence of *Alternaria alternata* infection on to capsaicin content of four Varieties of chilli

Variety	Capsaicin content (mg/100g)		
	Healthy	Diseased	% reduction
Pandurna	255.00	200.00	21.57
Pusa Jwala	425.00	214.00	49.65
C-1 Pant	382.00	255.00	33.25
Jawahar Mirch 218	415.00	303.65	26.83

It was observed that due to infection of *Alternaria alternata* the

capsaicin content was reduced , thereby reducing the quality of chilli fruits and directly affected the pungency which the major criterion of commercial chilli (Table 38).

16. Influence of infection by *A. alternata* on growth parameters of chilli plants.

Influence of infection by the fungus on plant height, number of fruits per plant, fresh weight of fruits, fruit length and girth of four varieties of chilli was determined.

16.1. Effect on plant height.

Chilli plant height was determined with the measurement scale at 130 and 160 day old crop. Twenty randomly selected healthy and diseased plants were measured. Infected plants were easily recognized under field conditions.

It is clear from the data presented in Table 39 that in all the four varieties of chilli grown in Goara farm Banda, diseased plants exhibited lesser height as compared to healthy ones. The height of healthy plants ranged from 43.8 to 59.8 cm at 160 days being maximum in Pandurna variety while it ranged 27.9 to 39.6 cm in diseased ones.

Table 39. Effect of infection of *Alternaria alternata* on the plant height of four varieties of chilli

Variety	Plant height (cm) at 130 and 160 days						
	Healthy		Diseased		% reduction		
	130	160	130	160	130	160	Average
Pandurna	37.0	59.8	31.5	39.6	14.86	33.78	24.32
Pusa Jwala	32.5	43.8	27.0	27.9	16.92	36.30	26.60
C-1 Pant	46.0	55.0	30.6	35.8	33.40	34.9	33.78
Jawahar Mirch 218	41.6	50.2	30.7	38.0	26.20	24.30	25.25

Maximum reduction of 33.4% was recorded in C-1 Plant plants infected with *A. alternata* at 130 days, while at 160 days it was 36.30% in Pusa Jwala. On the basis of average reduction, C-1 Pant, plants exhibited maximum reduction of 33.78%, followed by 26.60 % in Pusa Jwala, 25.25 % in Jawahar Mirch 218 and 24.32 % in pandurna (Table 39).

16.2. Effect on number of fruits and fresh weight of fruits

Influence of infection by *Alternaria alternata* on the number of fruits per plant and fresh weight of fruits was determined in four varieties and the results are presented in Table 40.

It is evident from the data that the diseased chilli plants had lesser number of fruits as compared to healthy. Maximum (39.9%) reduction in number of fruits was recorded in Pusa Jwala, followed by C-1 Pant (29.9% reduction). Least reduction was observed in Pandurna.

Similarly, the diseased fruits had lesser fruit weight as compared to healthy ones. Fruits of C-1 Pant variety were worst hit due to infection of *A. Alternata* where the weight of 25 healthy fruits was 64.0g as compared to 40.50g in diseased fruits. Pusa Jwala variety fruits had minimum disturbance due to infection and exhibited least reduction (13.64%) in fruit weight (Table 40.)

Table 40. Effect of infection of *Alternaria alternata* on the number of fruits per plant and weight of chilli fruits of four varieties.

Variety	No. of fruits per plant		% reduction	Fresh weight of 25 fruits (g)		% reduction
	Healthy	Diseased		Healthy	Diseased	
Pandurna	90.0	75.0	16.67	112.0	90.50	19.20
Pusa Jwala	105.0	64.0	39.9	66.0	57.00	13.64
C-1 Pant	101.0	71.0	29.90	64.0	40.50	36.72
Jawahar Mirch 218	101.0	82.0	18.80	110.5	89.00	19.46

16.3 Effect on length and girth of fruits

Effect on the length and girth of chilli fruits due to *Alternaria alternata* infection was determined and the results are presented in Table 41.

Maximum 30.56% reduction was recorded in Pusa Jwala while the length of healthy fruits was 8.90cm as compared to 6.15 cm in diseased ones (Table 41), followed by 30.33% reduction in Jawahar Mirch-218, 29.16 % in C-1 Pant and 13.08 % in pandurna. Similarly, the reduction in girth of fruits was observed due to the infection of *A. alternata*. Maximum 22.22 % reduction was noticed in Pandurna and minimum in Pusa jwala (13.33%) . Diseased fruits had lesser length and reduced girth as compared to healthy fruits.

17. Management of the disease

Alternaria alternata caused seed rot, seedling decay fruit rot and leaf spot diseases in chilli at different growth stages. Disease management was tried in two ways and the results are presented below.

Table 41. Effect of infection of *Alternaria alternata* on the length and girth of fruits of four varieties of chilli

Variety	Fruit length (cm)		% reduction	Fruit girth (cm)		% reduction
	Healthy	Diseased		Healthy	Diseased	
Pandurna	8.11	7.04	13.08	0.90	0.70	22.22
Pusa Jwala	8.90	6.15	30.56	0.75	0.65	13.33
C-1 Pant	7.75	6.00	29.16	0.85	0.71	16.67
Jawahar Mirch 218	9.80	6.80	30.33	0.86	0.70	18.60

17.1. Host Resistance

17.1.1. Evaluation of chilli varieties

Twenty-one chilli varieties were evaluated against *Alternaria alternata* infection under natural conditions of IGFR farm, Jhansi for two continuous years in 2002-03 and 2003-2004.

Disease index and reaction was recorded (Table 42) and it was observed that all the varieties growth both the years exhibited varied infection rate. None of the variety exhibited the desired resistance, however, kaliyanpur Yellow, G 3, SG 5, JCA181 , JCA 232, k 2, musalwadi and LCA 206 showed moderate resistance. In these varieties the disease index was upto 25. Minimum disease index 20.5 was observed in JCA 181 and maximum 24.5 in Musalwadi. It was interesting to note that kaliyanpur Red and some other varieties exhibited lesser disease index in 2002-03 and thereafter the disease index was increased in almost all the varieties (Table 42).

Table 42. Evaluation of twenty-one varieties against *Alternaria alternata* fruit rot infection under natural conditions of IGFR farm, Jhansi.

Variety	2002-03		2003-04	
	Disease Index	Disease Reaction	Disease Index	Disease Reaction
Pandurna	26.5	S	31.5	S
NP 46A	29.3	S	32.3	S
Pusa Jwala	29.7	S	36.7	S
Kaliyanpur Red	24.5	MR	29.2	S
Kaliyanpur Yellow	21.0	MR	24.0	MR
Bunchy	31.0	S	30.0	S
G 3	21.8	MR	22.5	MR
SG-5	16.3	MR	21.0	MR

C-1 Pant	32.5	S	39.5	S
JCA 31-B	31.0	S	32.3	S
JCA 111	30.5	S	33.0	S
JCA 181	21.0	MR	20.5	MR
JCA 208	25.0	S	39.0	S
JCA 232	21.5	MR	23.0	MR
Jawahar Mirch 218	33.3	S	31.3	S
NP 46	30.0	S	31.5	S
K 2	21.0	MR	24.2	MR
Musalwadi	19.2	MR	24.5	MR
LCA 206	21.5	MR	23.2	MR

17.2. Chemical management

17.2.1. Evaluation of fungicides against *Alternaria alternata* under laboratory conditions

Poisoned food technique was used for the evaluation of indofil M 45 (0.25%), Indofil Z-78 (0.25%), Fytolon (0.25%), Difolaton (0.25%), Triforin(0.15%), Captan (0.25%), Thiram (0.25%) and Derosal (0.15%).

The data presented in Table 43 indicate that all the fungicides tested under laboratory condition exhibited the inhibition in growth and colony diameter. Maximum inhibition was recorded in Triforin (0.15%) where the colony diameter was only 15.5 mm as compared to 86.5 mm in control, where no chemical was added to the medium. In Thiram (0.25%) only 25.5 mm growth was recorded, followed by 29.5 mm in Captan. Derosal (0.15%) and Fytolon (0.25%) showed least inhibition as compared to control.

Table 43. In vitro evaluation of fungicides against *Alternaria alternata* using poisoned food technique

Fungicide	% concentration	Radial growth (mm)	
		5 th day	8 th day
Indofil M 45	0.25	31.3	51.0
Indofil z-78	0.25	22.3	45.0
Fytolon	0.25	39.0	62.0
Difolaton	0.25	19.3	31.0
Triforin	0.15	6.2	15.5
Captan	0.25	17.3	29.5
Thiram	0.25	11.3	25.5
Derosal	0.15	53.0	77.5
Control	0.00	49.3	86.5

17.2.2. Efficacy of fungicides used as seed dresser

Eight fungicides were used as seed dresser. Seed sample No. 27 obtained from Pandurna was used in this experiment having maximum natural infection.

Observations on the association of *A. alternata* with chilli seeds indicate that in control 31.0 % seeds exhibited the infection of the fungus while its association was greatly reduced in all the treated seed, except in Derosal (29.0%) and Indofil Z-78 (13.0%).

The most effective combination was Thiram and Captan (1:1) where only 3.0% association was recorded, followed by 4.0% in Captan, 5.0% in thiram alone and 6.0% in Indofil M-45.

After fungicidal seed treatment the seed germination was improved (Table 44). Maximum (70.0%) germination was recorded in seeds treated with Thiram (0.25%) followed by combination of thiram and captan (1:1), captan (0.25%), 69.0 and 65.0%, respectively. In control only 38.0% seed germination was recorded.

Table 44. Efficacy of fungicides used as seed dresser against *Alternaria alternata* in naturally infected chilli seeds using standard Blotter Method

Fungicide	% concentration	% infection of A. alternata	% seed germination
Indofil M 45	0.25	6.0	42.0
Indofil z-78	0.25	13.0	42.0
Fytolon	0.25	11.0	55.0
Difolaton	0.25	7.0	62.0
Triforin	0.15	9.0	49.0
Captan	0.25	4.0	65.0
Thiram	0.25	5.0	70.0
Captan + Thiram (1:1)	0.40	3.0	69.0
Derosal	0.15	29.0	49.0
Control	0.00	31.0	38.0

17.2.3. Fungicidal Spray Trial

17.2.3.1. At Banda farm

Six fungicides, Indofil m-45, Indofil Z-78, Fytolon, Difolaton, Triforin and Derosal were applied three times starting from flowering to ripening. The data are presented in Table 45.

It is clear from the data that in all the treatments incidence of fruit rot incited by the fungus was comparatively very less. Minimum 5.8% fruit rot was observed in Triforin (0.15%) as compared to 31.5% in check where no fungicide was applied (Table 45).

Table 45. Influence of fungicidal application on the incidence of fruit rot of chilli incited by *Alternaria alternata* at during 2003-04 and 2004-05

Fungicides	% conce- ntration	% fruit rot incidence			% disease control over check	Yield of rip fruits (kg)
		2003- 04	2004- 05	Mean		
Indofil Z-78	0.25	10.5	9.5	10.0	68.25	15.5
Indofil M-45	0.25	6.5	9.3	7.9	74.92	18.3
Fytolon	0.25	8.5	6.9	7.7	75.55	15.7
Triforin	0.15	4.3	7.3	5.8	81.58	19.2
Difolaton	0.25	6.3	8.9	7.6	75.87	14.9
Derosal	0.15	21.7	29.9	25.8	18.09	12.5
Control	0.00	29.5	33.5	31.5	-	9.3

Maximum (81.58%) disease control was recorded in Triforin, followed by Difolaton (75.87%). Indofil m-45 (75.55%) over check. Minimum disease control was recorded in Derosal where 25.8% fruit rot was recorded as compared to 31.5% in control.

In Triforin 4.3 and 7.3% fruit rot was recorded during 2003-04 and 2004-05, respectively as compared to control (29.5 and 33.5%).

17.2.3.2. At IGFRI farm, Jhansi

Five fungicides, Indofil M-45, Indofil Z-78, Fytolon, Triforin and Difolaton were used and applied three times from flowering to ripening. The data presented in Table 46 indicate that minimum (10.3%) fruit rot incidence was recorded in Triforin, followed by 10.5% in Indofil M-45 as compared to control (27.3%). Maximum disease control was recorded in Triforin (62%), followed by 61.53% in Indofil M-45. Indofil Z-78 showed least disease control (Table 46).

Indofil M-45 (0.25%) and Triforin (0.15%) exhibited maximum disease control at Banda and IGFRI.

Table 46. Effect of fungicidal application on the incidence of fruit rot of chilli incited *Alternaria alternata* at IGFRI during 2003-04

Fungicides	% concentration	% fruit rot	% disease control
Indofil M-45	0.25	10.5	61.53
Indofil Z-78	0.25	14.3	47.61
Fytolon	0.25	12.3	54.94
Triforin	0.15	10.3	62.27
Difolaton	0.25	13.5	50.54
Control	0.00	27.3	-

DISCUSSION



DISCUSSION

Fruit rot, leaf spot and drying of tender tips caused by *Alternaria alternata* (Fr.) Keissler is a major problem limiting profitable cultivation of chillies (*Capsicum annuum* L.) The disease appears every year in the region wherever the crop is cultivated and often assumes serious proportions under favourable environmental conditions. The disease is of great importance in Uttar Pradesh (as it causes heavy loss to the crop in quantity and quality of the produce.)

Bundelkhand region of Uttar Pradesh is a dryland area having sub-tropical climate with hot dry summer and cold winters and annual rain fall about 700mm.

Red and green chillies have an important role in our daily diet. No Indian dish is complete without chillies being an indispensable condiment. Introduced by Portugese in India, chillies are the major and cheapest source of Vitamin C and A (Singh, 1989). It is a unique crop among all the spice crops being the only source of capsaicin ($C_{18}H_{27}NO_3$), an alkaloid the active hot principle which is a mixture of seven closely related alkylvanillylamides named capsaicinoides (Rogers, 1966, Maya, 1975, Govindrajan, 1985, Tiwari, 1990). The red colour of the fruit is due to the pigment capsanthin (Nath, 1969). Chilli fruits are used as irritant for rheumatism or neuritis. Presence of rutin in the fruits provides a unique medicinal value (Purseglove, 1977 and Singh, 1989).

With a view to understand the distribution and occurrence of major diseases of chilli, a pilor survey was undertaken in 2002 and 2003 at five locations and 10 diseases were recorded affecting the crop at various crop stages. Wilt incited by bacterium *Pseudomonas solanacearum* and fungus *Fusarium spp.* is a widespread problem exhibiting 42.5% plant infection

at Bisanda gram, whereas the ripe fruit rot and leaf spot incited by *Colletotrichum dematium* ranged from 11.0 to 19.1%, followed by leaf spot by *Cercospora sp.* (12.1%). Among the viral diseases, mosaic ranged from 6.2 to 13.0%. *Sclerotinia* wilt and powdery mildew, little leaf, *Phytophthora* blight and root-knot were in traces. Fruit rot and leaf spot incited by *Alternaria alternata* was observed in all the five locations ranged from 8.5 to 19.5% at Tindwara and Bisandagram, respectively. The *Alternaria* fruit rot and leaf spot was considered to be the major problem and ranked third to wilt and ripe rot. In fact, so far, major thrust and attention was paid to first two diseases and fruit rot and leaf spot by *Alternaria sp.* was ignored, hence very little information is available about the disease.

For systematic and detailed information, a survey was conducted during 2002, 2003 and 2004 around Banda covering 20 locations during September to February. Per cent disease incidence was calculated by counting the infected plants and fruits in a particular unit area which was divided by the total number of plants observed multiplied by one hundred. On the basis of average of three years observations maximum fruit rot 21.4% was noticed at Kanwara, while maximum twig drying (18.0%) was noted at Jari. The crop is cultivated either as a mixed or sole crop in the region, however, no variation and influence of cropping system was observed.

In Bundelkhand region maximum cultivated area for chilli is in Jhansi district. During 2003 and 2004 around Jhansi, 15 locations were visited for the occurrence of the disease. Maximum 17.2% and 17.2% twig drying was recorded at Hansari and Chirgaon respectively. Maximum 23.3% fruit rot was noted at Karari.

During 2002-03, 5 locations were visited and it was conclusively established that the disease is widespread, appearing every year and assuming serious proportions.

It was also recorded that disease is more prevalent during January/February as compared to September/October crop.

The prevalence of the disease in the region has been recorded by Mishra (1963), Harne and Name (1967), Chaurasia (1976), Jharia *et al.*, (1977), Verma and Bhale (1989). In India, the disease was recorded by several workers (Edward and Srivastava, 1954; Mathur and Agnihotri, 1961; Singh and Tandon, 1967; Sreekantiah *et al.*, 1973; Manoharachari and Padmavati 1976; Mali and Joi, 1985; Singh, 1987; Sujatha Bai *et al.*, 1993, b).

During survey diseased chilli fruits, stem, twig, and seeds were collected, examined for the association of *Alternaria alternata*. Upon repeated isolations on potato dextrose agar medium, the fungus was constantly found associated with infected plant parts. Several isolates were compared and no critical variation was noted. Identification of the fungus was made on the basis of morphological and cultural characters described by Ellis (1971) and Subramaniam (1971). *Alternaria alternata* (Fries) Keissler is the correct valid name for *Alternaria tenuis* (Nees) which is generally characterised by long conidial chain, marked polymorphic conidia according to International Rules for Nomenclature (Simon, 1967; Lucas, 1971).

Upon repeated isolates on PDA the fungus was recorded. Purification of the fungus was done by hyphal tip method and single spore isolation method as described by Ezekiel (1930). Colony of the fungus on PDA was light grey, later turning black, ultimately olivaceous green-black. The mycelium was aerial, abundant, varying from fluffy cottony to closely tufted. Hyphae were branched, 3-4 μ in thickness.

Conidiophores were simple or branched, erect, septate, geniculate often with scars and swellings. Conidia were light brown, olive, smooth, muriform with 3 to 6 transverse septa 1 to 3 longitudinal septa. Conidia were variable in shape, size measured from 13.2-42.8 x 10.6 to 13.2 μ . They were in chains and with single beak.

The morphological and cultural characters of *Alternaria alternata* have also been described by Gupta and Das (1964); Rao (1965); Mukhopadhyay (1969); Dhanraj (1970); Singh *et al.* (1980); Singh *et al.* (1982); Singh and Suhag (1983), Haware *et al.* (1986).

Symptoms incited by *Alternaria alternata* have been recorded critically in this investigation on chilli seeds, seedlings, leaves, twigs and fruits. Infected seeds were smaller in size, rusty brown to black and in severe cases shrinkage was recorded. In badly infected fruits the seeds just beneath the pericarp were black and covered with mycelia growth. Seed rot was also recorded. Infected seeds could not germinate as observed in standard blotter method and growing-on test in soil. Brownish sunken discolouration of seedlings in severe infections coverage with mycelial growth was also observed. Infected seedlings often toppled down. Progressive downward necrosis of tender tips of chilli plants was noticed during December/January. The dead twigs became silvery-white in due course of time. On stem, black brown specks were also recorded. On leaves, brown spots producing target-board-effect symptoms were common with narrow chlorotic margin which faded into normal green and increased with increase in size of spot. The pericarp of infected chilli fruit became greenish-brown with irregular brown spots which ultimately turn velvety due to moldy growth on the fruits. Badly infected fruits become straw coloured.

Symptoms produced on different plant parts are in close agreements to those described by Sibia (1957); Courter and Shurtleff

(1965); Tutunaru and Raicu (1970); Alam *et al.* (1981); Sujatha Bai *et al.* (1993a).

During the present investigation it was recorded that the pathogen can attack at all the stages of crop growth. The infection on seed, seedling, ripe and semi-ripe fruits was recorded while Sujatha Bai *et al.* (1993a) did not find infection on semi red and small green fruits. Uma (1981), observed that the infection intensity was higher with ripe fruits. Seed rot and seedling decay was also recorded during the investigation which is again in close agreement to the observations made by Edward and Shrivastava (1954) and Walter *et al.* (1974). The pathogen has also been recorded as internal mould in sun dried chillies (Leyendecker, 1954a, Spalding and King, 1981).

The virulence of the isolated fungus was tested by seed-infection, soil infection and fruit inoculation methods. Upto 63.0% rotting of chilli seed was recorded when the healthy chilli seeds were infested with actively grown pure culture of *Alternaria alternata* and plated on blotters for incubation. The uninjured seeds exhibited only 45.0% infection. Artificially injured seeds proved more vulnerable to infection and had lesser germination percentage, The results are in close agreement with Sultana *et al.* (1988).

Seed infestation method with *Alternaria* spp. was also found useful by Singh *et al.*, (1977) while working with sunflower and Hyde and Galleymore (1951) with wheat grains.

In soil infestation method, mycelial mat was mixed in the soil and seed free from natural infection were sown. Pre-emergence rot upto 29.0% and post-emergence rot 11.0% was noticed after 19 days of incubation in pot house. The association of the fungus was confirmed by plating the infected portions on PDA which yielded the fungus. Changri

and Weber (1963) also proved the pathogenic behavior of *Alternaria* by soil infestation method for cruciferous seeds.

Fruits of Cv. Pusa Jwala were used for testing the ability of *Alternaria alternata* to infect it. Semi ripe and ripe fruits were inoculated by pin prick method, spore suspension spray method, carborandum rub method and tooth pick prick method. Pin prick method was the best on chilli leaves, semi ripe and ripe fruits. Symptoms appeared within 7 and 9 days on intact and detached chilli fruits. It was also noted that ripe fruits were more vulnerable to infection than semi ripe ones. Uma (1981) and Sujatha Bai *et al.* (1993a) also reported the higher association of the fungus with matured fruits.

Alternaria alternata has a wide host range and is capable of causing diseases on lentil (Gupta and Das, 1964), pea and cowpea (Rao, 1965), mungbean (Gupta, 1970), sunflower (Singh *et al.*, 1977), sunflower (Singh and Chand, 1982), sesame (Yu *et al.*, 1982), chickpea (Haware *et al.*, 1986) and so many others.

Association of *Alternaria alternata* and other mycoflora with chilli seeds was observed by standard blotter method (ISTA, 1985). In all, 50 seed samples were collected and tested. Twenty seed samples were collected during survey one sample from each location, eleven seed samples obtained from IVRI, Varansi, thus making 31 seed samples and seed samples of 19 varieties/lines were procured from Department of Horticulture, CSAUAT Kanpur. The association of mycoflora was observed on seeds untreated as well as pretreated with 0.1% mercuric chloride. In all, 17 fungi were found associated which included *Alternaria alternata*, *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Curvularia lunata*, *Curvularia* sp., *Chaetomium* sp., *Cladosporium oxysporum*, *Collectotrichum capsici*,

Dreschlera tetramera, *Fusarium oxysporum*, *Fusarium solani*, *Memnoniella* sp., *Penecillum* sp. and *Rhizopus* sp.

Seed-borne mycoflora of chillies has been studied by Suryanarayana and Bhombe (1961), Manoharchari and Padmavati (1976), Chaurasia (1976), Siddiqui *et al.*, (1977), Dhawale and Kodamelwar (1978), Hashmi (1989), Dhyani *et al.* (1990), Padaganur and Naik (1991).

The association of *Alternaria alternata* ranged from 2.0 to 32.0%. The association of other fungi is as follows: *Aspergillus flavus* (1.0 to 15.0%), *A. niger* (1.0 to 18.0%), *A. fumigatus* (1.0 to 8.0%), *Botrytis cinerea* (1.0 to 11.0%), *Curvularia lunata* (1.0 to 16.0%), *Curvularia* sp. (1.0 to 12.0%), *Chaetomium* sp. (1.0 to 4.0%), *Cladosporium oxysporum* (1.0 to 8.0%), *Colletotrichum capsici* (1.0 to 29.0%), *Dreschlera tetramera* (1.0 to 12.0%), *Fusarium oxysporum* (1.0 to 11.0%), *Fusarium solani* (1.0 to 8.0%), *Memnoniella* sp. (1.0 to 10.0%), *Penecillum* sp. (1.0 to 9.0%), *Rhizopus* sp. (1.0 to 12.0%). The association of *Alternaria* sp. ranged upto 11.0%.

Seeds of 19 varieties/lines were also tested for the seed-borne mycoflora and 14 fungi were found associated. Pretreated chilli seeds exhibited lesser association than untreated seeds, reason being the externally seed-borne fungi were eliminated due to the treatment, similarly increased germination fo seeds was noticed in pretreated seeds.

Based on the maximum natural infection of *A. alternata* four seed samples were selected for further studies. The seed samples were from Jhansi (Sample No. 21 having 28.0% natural association), Hamirpur (Sample No. 24 having 28.0% association), Mahoba (Sample No. 27 having 30.0% association) and Banda (Sample No. 30 having 32.0% association).

Apparently healthy and rusty brown shriveled chilli seeds were classified by naked-eye examination and were tested for the association of mycoflora by Standard Blotter Method (ISTA, 1985). Association of *A. alternata* was 28.5% in apparently healthy bold seeds as compared to 29.5% in case of shrunked rusty seeds. In all, ten fungi were recorded. Apparently healthy seeds had lesser number of fungi as compared to shrunked seeds. Germination of seeds was higher in apparently healthy seeds as compared to rusty seeds. The relation of apparently healthy seeds and shrunked seeds for the association of mycoflora was studied in soybean (Chacko, 1971), sesame (Kushi, 1971), urid bean (Singh, 1977), jowar (Prasad, 1982). It appears very essential to process the seeds before planting so that damaged, shrivelled, rusty and uneven seeds may be removed and risk of inoculums build up can be minimized.

Standard Blotter Method, Agar plate method, 2, 4-D method and Deep freeze method were used for the detection of *Alternaria alternata* associated with chilli seeds and for other mycoflora as suggested by ISTA (1985), Musket and Colhoun (1948), Limonard (1968), Neergaard (1973). Four seed samples of chilli having maximum natural infection of *A. alternata* were used. Modifications of standard blotter method, Agar plate method, 2, 4-D method and Deep freeze method were also tried. In this way, the detection of *Alternaria alternata* with four selected seed samples was tried by 12 methods. Comparative efficacy of different methods revealed that Standard Blotter Method (ISTA, 1985) was the best in which maximum 31.0% association was recorded of *Alternaria sp.* associated with chickpea and sesame. This method has also been used by Singh *et al.*, (1977); Singh *et al.*, (1980); Yu *et al.*, (1982) in sunflower and sesamum. Mishra (1963) and Harne and Nema (1967) also recorded the Standard blotter method good for the detection of *Alternaria sp.* associated with vegetable seeds. Shah and Jain (1993) found Standard

blotter method superior over Agar plate method in the detection of *Alternaria alternata* associated with mustard seeds, and by Jain and Patel (1969) in case of castor.

Seed-borne nature of *Alternaria alternata* with *Capsicum annuum* has been demonstrated by Suryanarayana and Bhombe (1961), Harne and Nema (1967), Rout and Rath (1972), Walter *et al.* (1974), Chourasia (1976), Manoharachari and Padmavati (1976) and Siddiqui *et al.*, (1977). Various factors govern the growth sporulation of a fungus. In the present investigation ten synthetic and non-synthetic media were used and maximum colony diameter (86.0mm) was recorded in potato dextrose agar medium after eight days incubation, followed by chilli seed extract agar and Richard's agar. Bhargava *et.al.*, (1992) recorded potato dextrose agar to be the best medium for chickpea isolate of *Alternaria alternata*. Fahim (1966) reported PDA to be superior for the isolation of *A. porri*. Superiority of PDA was also recorded by Singh and khanna (1969), Hasan (1970), Siddaramaiah and Hegde (1984) for the isolation of *Alternaria alternata*. Among other nonsynthetic media, oat meal agar was the best and reported by Mehta and Prasad (1976) for *Alternaria* sp. isolate of sesame, whereas Pero and Main (1970) found favourable growth of *Alternaria tenuis* on autoclaved rice grains supplemented with yeast extract.

To find the best temperature and hydrogen ion concentration (pH) for the growth of *Alternaria alternata*, four levels of temperature and eight levels of pH were tried. It was recorded that fungus grew best at 30°C with maximum (91.0mm) colonydiameter, followed by 25°C. Maximum spores (34per microscopic field) were recorded at 30°C. Bhargava *et al.* (1992) found 25°C best for the growth of *Alternaria alternata*. In the present investigation maximum (91.00mm) diameter of the colony growth was recorded at pH 6.5. Arya and Prasad (1952)

indicated that pH 3.0 to 8.5 range was suitable for the growth, while Mohanty *et al.*, (1981) also found pH 6.0 to be the best. Samual *et al.*, (1971) found optimum pH 5.0 and temperature 24-36°C, whereas Hasan (1970), reported optimum temperature 25°C and pH 5.4 for the fungus. Jamaluddin and Tondon (1975), recorded the best mycelial growth at pH 7.0.

Spore germination is a prerequisite for infection of host by a fungal pathogen. According to Wolf and Wolf, (1947) spore germination is influenced by two distinct group of factors viz. heredity or internal and environmental or external factors. Among the environmental factors, moisture, temperature, pH, nutrient, aeration and presence of stimulating or inhibiting substances have been reported to exert a profound influence on this process. In the present investigation, it was observed that spores of *Alternaria alternata* started germination within 4:30 hours at 22°C. Unipolar, bipolar and septal germination was noted. The spore germination took about 6:30 hours at 15°C. 5:30 hours at 30°C and 5 hours at 25°C, while studying the influence of four levels of humidity, it was observed that at 90% relative humidity germination started within 6:00 hours and was completed in 18 hours and, while at 75% the germination started within 12 hours and only 61.0% germination was noted. At 25% relative humidity no spore germination was observed even after 18 hours.

Spore germination process of *Alternaria spp.* isolated from various hosts has been studied by Chaudhary (1944), Pawar and Patel (1957); Berry (1960); Bock (1964); Mohapatra *et al.* (1978); Rajpurohit and Prasad (1982). Average temperature found suitable for germination was 25 to 30°C. observed that spore germination of *Alternaria sp.* was best at 25 to 30°C. Pawar and Patel (1957) observed that spore germination of *Alternaria sp.* was best at 25 to 30°C, while Bock (1964) reported it to be

30°C. Verma (1987) concluded that spores of *Alternaria carthami* germinated at 30°C at pH 5.0 Chaudhary (1944) also concluded that pH 6.0 was the best for the growth and pH 5.0 for spore germination of *Alternaria carthami*.

Influence of exogenous supply of carbohydrate as sucrose was tested and five concentrations, 0.01, 0.25, 0.5, 1.0 and 1.5% were used and effect on spore germination of *Alternaria alternata* was recorded. Accelerated spore germination was noted. It was interesting to note that at 0.01 and 0.25% complete germination was achieved within 3 hours as compared to 5 hours in control.

The liberation of chemicals during seed germination has been reported which have inhibitory or stimulatory influence on fungal spore germination (Schroth and Synder, 1961; Agrawal and Khare, 1974; Shukla, 1974; Naim *et al.*, 1976). In the present investigation the possibility of such process was studied. In the present investigation it was observed that seed exudation has inhibitory effect on spore germination of *Alternaria alternata*. In chilli seed exudated suspension 72% spore germination was recorded as compared to 96.0% in control.

The knowledge of disease cycle of a fungus is very essential to located the weak links which may be attacked for better disease management. Disease cycle of *Alternaria alternata* causing leaf spot, fruit rot and tender tip drying of chilli was not fully studied, however Agrios (1988) has attempt to compile information on this aspect.

In the present investigation several experiments were conducted to study the transmission of the fungus from seed to plant and plant to seed. During survey 50 seed samples were collected., grouped locality and variety-wise and tested for the association of *Alternaria alternata* by Standard blotter method and Standard agar plate method. The seeds were surface sterilized with 0.1% mercuric chloride. This provided better

chance to internally seed borne fungi to develop. Pretreated and untreated seeds of chilli revealed the association of the fungus indicating both external and internal association of the fungus indicating both external and internal association. Maximum (32.0%) association of the fungus was recorded in a seed sample obtained from Mahoba (Seed sample No. 27). Seed-borne nature of *Alternaria alternata* with *Capsicum annuum* has been reported by several workers (Nobel and Richardson, 1968; Suryanarayana, 1978; Rout and Rath, 1972; Manoharachari and Padmavati, 1976).

Seed to plant transmission of the fungus was studied by two methods, water-agar-seedling symptom test (Khare et al., 1976) and growing on test in sterile sand (ISTA, 1985). Total 38.0% seed rot and seedling mortality due to *Alternaria alternata* was recorded. The seed and seedling infection was confirmed by isolation of the fungus on potato dextrose agar medium. In growing on test, when the seeds were sown in sterile sand soil in plastic pot 28.0% seed rot and 9.0% seedling infection was confirmed. Increased infection in seed and seedling confirmed the movement of the fungus. Seed to seedling transmission of *Alternaria sesamicola* was demonstrated and confirmed by Singh *et al.* (1983) by using growing on test. Systemic nature of the fungus was also reported.

Site of infection of *Alternaria alternata* was observed by seed component plating, through microscopic observations of hand-cut sections of seeds and histopathological studies. Exact components of chilli seed could not be plated due to very fragile nature, however the fungus could be observed externally and internally seed-borne. Observations under compound microscope on the cross section (hand-cut sections) revealed the presence of fungal remnants. In the distorted portions of seed where large cavity was formed and fungal mycelium was recorded.

Histopathological studies of infected leaves revealed the disintegration of tissues in progressive manner. The fungal hyphae were seen in the distorted portion. In case of studies of infected stem due to fungal infection, disintegration in cuticular, epidermal and hypodermal tissue was noted; however, no mycelium was recorded in pith and cortical tissues.

Plant to seed transmission of *Alternaria alternata* was studied by (i) fruit inoculation technique and thereafter isolation of the fungus from each part and (ii) plant samples were also collected from different places and association of the fungus was noted by microscopic examination and subsequently by isolation on PDA. The chilli fruit of three different stages of growth, green, green-yellow, and yellow-red were inoculated with hypodermic needle injecting with 3ml spore suspension. Seeds were harvested and tested. It was observed that after harvesting the fruit inoculated at yellow red stage exhibited 72.0% infection, green yellow stage 67.0% and green stage 40.0%. In yellow red stage infection, seed formation was not normal seeds were somewhat shriveled and deformed. The components of artificially inoculated fruits were also plated to confirm the association. In all the stages of fruits and all the components viz., pericarp, seed, septum and placenta were found infected. In green stage fruits septum part did not show any infection. Similarly no association of the fungus was observed in calyx portion. It was concluded that the fungus penetrated all the fruit parts and moved to the seeds. When yellow-red fruits were cut open, fungal mycelia growth was recorded just beneath the pericarp infecting the seeds. During the survey, infected plant parts, chilli fruits, seeds, twigs, stem and leaves were collected and used for isolation on PDA. The fungus was found to be associated with all the parts collected during visits. In the present investigation, green fruits were also found susceptible to the infection of

A. alternata contradicting the findings of Sujatha Bai *et al.* (1993a). They did not find infection on green chilli fruits. Mridha and Siddiqui (1989) have found direct correlation between fruit infection and subsequent seed transmission of *A. alternata* in chilli.

Survival of the fungus was tested by plating the portions of twig and fruits stored in paper envelopes and mixed in sterile sand/soil as plant debris. Periodical isolation from the material kept in envelop and in soil and subsequent growth observed on potato dextrose agar medium indicated the viability of the fungus. The fungus could be isolated upto 120 days from the infected twigs, 150 days from fruits and 210 days from seeds kept in the paper envelopes at ordinary room temperature and checked at 30 day interval. In soil, isolations could be made upto only 120 days. Initially the viability was only 70.0% and after 120 days 10.0% only. There are very few reports on the period and mode of survival of *Alternaria alternata*. Bharava *et al.* (1992) observed that the fungus could survive saprophytically in the plant debris left in the field. They found that the inoculums gets reduced to 10.0% on seed and 30.0% in debris upto next sowing time in chickpea. Survival of the fungus has also been studied by Laszolo (1969) and Suhag and Singh (1989). Basu (1971) recorded the survival of *Alternaria porri* in the form of *chlamydospores* while, Pandotra (1965) reported the survival in the form of spores and mycelium in plant debris.

The concept that plant pathogens cause diseases by producing toxic substances dates about a century back. Evidence for its general validity has been discussed by Owens (1969), Ciegler *et al.*, (1971). *Alternaria alternata* produces tentoxin which causes general chlorosis (Agrios, 1988). In the present investigation, production of toxic metabolites was confirmed by the fungus. It was noted that seeds soaked in filtrate of 5, 10, 15, 20, 25 and 30 day old culture showed inhibition in seed

germination. Maximum reduction in seed germination was 70.24% when the seeds were soaked in culture filtrate from 15 day old culture. Studies on the influence of dilutions of the culture filtrate indicate that in crude culture filtrate the seed germination was only 20.0% as compared to 84.0% in filtrate obtained from uninoculated medium and 86.0% from distilled water, as the filtrate was diluted the increase in seed germination was observed. Similarly the elongation of root and shoot was adversely affected by crude culture filtrate and its different dilutions. The root length (3.0mm) and shoot length (2.0mm) was recorded when subjected to crude filtrate as compared to 65.0 mm and 34.0mm in distilled water, respectively.

It was observed that soaking period has a positive role in absorption of toxic material. The chilli seeds were soaked in culture filtrate for 1, 2, 3, 6, 9 and 12 hours. Seeds soaked in the culture filtrate for more than 3 hours did not germinate. Seeds soaked in culture filtrate for 1, 2, 3 hours exhibited 18.0, 24.0 and 26.0% seed germination as compared to 63.0 to 73.0% when the seeds were soaked in either distilled water or Richard's medium filtrate.

Influence of temperature on the activity of toxic metabolite was observed in the present investigation. It was concluded that when the filtrate was heated upto 50°C it exhibited comparatively lesser seed germination (30.0%) as compared to distilled water treated seeds (87.0% seed germination).

The toxin production of *Alternaria spp.* Has been discussed by several workers (Fulton *et al.*, 1965; Templeton *et al.*, 1967; Pero and Main, 1970; Meronuck *et al.*, 1972; Singh, 1978; Siddaramaiah *et al.*, 1984; Gareth, 1987). The ten toxin produced by *Alternaria alternata* is host non-specific and effective even at 2 ppm. It is a cyclopeptide with molecular formula of $C_{24} H_{32} N_4 O_4$. Tenuazonic acid, alternariaol

monomethyl ether was isolated from tentoxing. Progressive inhibition of seed germination, root elongation and production of host non-specific toxin has been observed by Meehan and Murphy (1947), Sahni et al. (1974). In the present investigation, bioassay method based on the inhibition of root elongation and toxicity determination in terms of per cent inhibition of seed germination as suggested by Narain and Das (1976) was adopted.

Aust (1986) has enumerated several factors responsible for disease development which include fog and dew, temperature, humid weather, dense mist and precipitation in the form of rains. In the present investigation, influence of temperature, humidity levels and age of chilli fruits was determined on the development of rot incited by *Alternaria alternata*. It was recorded that maximum (55.0%) increase in the rot was observed in Pusa Jwala at 25°C while it was 49.0% in Jawahar Mirch-218. In both the varieties, at lower temperature (10° and 15°C) development was less.

In Pusa Jwala maximum (48.0%) increase in fruit rot was observed at 100% humidity level being 38.0% after 10 days and 86.0% after 15 days of incubation. In Jawahar Mirch-218 maximum fruit rot (80.0%) was recorded at 80% relative humidity while maximum increase was 39.0% at 90% relative humidity.

The influence of age on the development of rot was determined and it was concluded that as the age increased the rotting also increased, however, tarred ripe stage the infection rate was comparatively lower. Yellow red stage was most susceptible as compared to green yellow. Similarly green stage was less susceptible as compared to red stage. Young fruits were less susceptible.

Influence of temperature and humidity on the development of disease incited by *Alternaria spp.* In different crops has been discussed by

several workers (Bock, 1964; Pandotra, 1964; Treggi and Rainaldi, 1966; Fahim, 1966; Boelema and Ehlers, 1967, Rotem, 1978). Verneau (1959); and Mathur and Agnihotri (1961) realized the importance of temperature in the development of dry weather on fruit rot of chillies. Increased disease development in high humidity was noticed by Bhargava and khare (1988a) in chickpea, while Suhag *et.al.*, (1985) also discussed the role of humidity and temperature in *Alternaria sp.* disease of radish. The role of temperature and humidity in safflower leaf spot caused by *Alternaria carthami* has been discussed by Fahim (1966); mc Rae *et al.*, (1984). The results of present investigation are in close agreement with the findings of these workers.

Fungal infection results in great shifts in the nutritive content of host tissues. Effect of infection of *Alternaria alternata* on ascorbic acid, carotene and capsaicin was determined on naturally infected fruits of green, fresh red turning and red chillies. Reduction in ascorbic acid was noticed at all the stages in all the four varieties. Maximum 41.85% reduction was noticed in C-1 Pant, followed by Pandurna (36.45%). Jawahar Mirch 218 (23.29) and Pusa Jwala (21.66%). It was interesting to note that fruits of C-1 Pant contained maximum ascorbic acid (302.00mg/100g) among all the four varieties. It was also observed that as the fruits ripened the ascorbic acid content lowered down but remained higher as compared to green ones. Similarly, the carotene content of diseased and healthy fruits was also varied and it ranged from 0.165 mg /100g to 0.183mg in healthy as compared to diseased from 0.083 to 0.132 mg /100gm. Maximum reduction in carotene content of Pusa Jwala (53.89%) was recorded, followed by 44.51% in Jawahar Mirch-218.

The chillies are valued for their pungency throughout the world and it is due to an alkaloid. Maximum reduction (49.65%) in capsaicin content was noted in Pusa Jwala (425.0 as compared 214.0mg/100g in

healthy and diseased, respectively) while minimum in Pandurna (21.57%). The capsaicin content reduction ranged from 21.57% to 49.65%.

Due to the infection of *Alternaria alternata* the reduction in nutritive contents like ascorbic acid, carotene and capsaicin was observed in the fruits of chillies in the per sent investigation. The pungency of chillies is due to the presence of an alkaloid capsaicin. This hot active principle of chillies consists of seven closely related alkylvanillamides named capsaicinoides (Tiwari, 1990). The ovary wall of chilli fruit is responsible for production of capsaicin while placental cells contain maximum alkaloid and the pericarp has smaller amount.

The reduction in ascorbic acid during pathogenesis of four tomato fruit rot fungi, *Phoma exigua*, *Rhizoctonia solani*, *Stemphylium vesicarium* and *Nigrospora oryzae* were studied by Reddy et al, (1980).

Rapid decline in ascorbic acid during the pathogenesis of chilli fruits due to *Choanephora cucurbitanum* was noticed by Chahal and Grover (1972). Reduction in amino acid content was also recorded by Shivaprakasan et al. (1972).

Awasthi and Singh (1975) observed the decrease in capsaicin content and ascorbic acid in chilli fruits infected with cucumber mosaic virus, while changes in the nutritive content due to *Colletotrichum* sp. infection was recorded by Azad (1991).

Influence of infection by *Alternaria alternata* on plant height, number of fruits, fruit weight, length and girth of fruits was recorded. Overall reduction in agronomical aspects was recorded due to the fungal infection in all the four varieties. Maximum reduction in plant height 33.4% was recorded in C-1 Pant plants at 130 days, while at 160 days it was 36.3% in Pusa Jwala.

The diseased plants had lesser number of chilli fruits. Maximum reduction (36.3%) was recorded in Pusa Jwala. Least reduction was in Pandurna variety. Similarly, the diseased fruits had lesser weight as compared to healthy ones. Fruits of C-1 Pant were worst hit.

The fungal infection also adversely affected the length and girth of fruits. Maximum reduction 30.56% was in Pusa Jwala (8.90 cm as compared to 6.15 in diseased fruits). Maximum reduction (22.22%) in chilli fruit girth was noticed in Pandurna. Diseased fruits had lesser length and reduced girth as compared to healthy ones.

Chilli crop has been observed to suffer due to seed and seedling rot, leaf spot, fruit rot and tender tip drying incited by *Alternaria alternata*. In the present investigation, attempts were made to find suitable control measures for the disease by germplasm evaluation, fungicidal evaluation under laboratory conditions, seed dressing chemicals and foliar spray trials under natural field conditions.

In the present investigation, 21 chilli varieties were evaluated under natural conditions of IGFRI, Jhansi during 2002-03 and 2003-04 for two continuous years. Although none of the variety exhibited the desired resistance; however, Kaliyanpur Yellow, G 3, SG 5, JCA 181, JCA 232, K2, Musalwadi and LC 206 showed the moderate resistance. Minimum disease index (20.5) was observed in JCA 181, and maximum (24.5) was in Musalwadi. Search for management of disease through selections and breeding of different genotype has also been a major area of research in the management strategies. At Hissar, Chauhan and Duhan (1986) evaluated 87 strains/varieties of Capsicum against anthracnose, *Alternaria* blight, *Cercospora* leaf spot and leaf curl. Germplasm evaluation against *Alternaria* sp. has been done by Melikova (1960); Courter et al. (1965); Verma and Bhale (1989) and Sujatha Bai et al. (1993b).

In the present investigation, eight commercially available fungicides were evaluated by poisoned food technique. Maximum inhibition of the growth of *Alternaria alternata* was recorded in Triforin (0.15%) where the colony diameter was only 15.5mm as compared to control (86.5mm). Triforin (N, N'-bis (1-formamido-2, 2, 2-trichloro ethyl)-piperazine) was the most effective, while Derosal (Methyl-2-Benzimidazole carbamate) was found to be least among the eight fungicides tested in vitro.

Seed-borne nature of the fungus has been recorded by several workers. The use of chemicals as seed-dressing is recommended by several workers for the reduction of load of seed mycoflora, including *Alternaria* sp. (Suryanarayana, 1978; Agrawal and Sinclair, 1987; Mridha and Chaudhary, 1990).

In the present investigation, the most effective combination was of Thiram and Captan (1:1) where only 3.0% association of *Alternaria alternata* was observed, followed by Captan (4.0%) as compared to 31.0% in control. The fungicidal seed treatment not only reduced the association of the fungus but also increased the seed germination. Efficacy of Thiram + Captan (1:1) has also been found effective by Jharia *et al.* (1977). Reduction in the seed borne *Alternaria spp.* by the use of chemical has been observed by Crisan and Merescu (1970); Ellis *et al.*, (1977); Tutunaru and Raicu (1978); Kannaiyan *et al.*, (1980); Siddaramaiah *et al.*, (1980); Mali and Joi (1985); Dhyani *et al.*, (1990); Mridha and Chowdhury (1990).

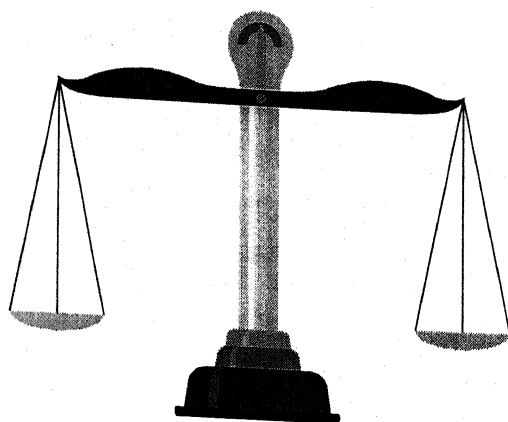
The fruit rot and leaf spot due to the fungus have also been recorded. In the present investigation, under natural field conditions of Banda, 6 fungicides were evaluated for the control of the disease. The fungicides were applied three times starting from flowering to ripening. Maximum (81.58%) disease control was achieved in Triforine (0.15%),

followed by Difolatan (75.87%), Indofil M-45 (75.55%) over check. Minimum (5.8%) fruit rot was observed in Triforine as compared to 31.5% in check where no fungicide was applied. In Triforine (0.15%) 4.3 and 7.3% fruit rot was recorded during 2003-04 and 2004-05, respectively as compared to control (29.5 and 33.5%).

Similarly at IGFRI, Jhansi five fungicides were evaluated under natural field conditions and Triforine (0.15%) was found the best, followed by Indofil M-45 (0.25%). Minimum (10.3%) fruit rot incidence was recorded in Triforine, followed by 10.5% in Indofil M-45 as compared to control (27.3%).

Jaria *et al.* (1977) recorded four sprayings of Zineb, (0.25%) very effective against foliar diseases which include infection by *Alternaria* sp. while efficacy of yellow copper oxide was observed by Brauer and Richardson (1957). Chemical control studies have also been undertaken by several workers (Leyendrcker, 1954a; Shurtleff and linn, 1963; Singh and Tondon, 1967; Perwaiz *et al.*, 1968; and Rahman *et at.*, 2005).

CONCLUSION



CONCLUSIONS

Chilli (*Capsicum annuum* L.) is an important indispensable condiment as well as a vegetable crop. It is a unique crop being the only source for capsaicin and the major source for Vitamin C and A.

Alternaria alternata (Fries) Keissler has been identified a major problem limiting profitable cultivation of chilli (*Capsicum annuum* L.) and is responsible for seed rot, seedling decay, leaf spots, fruit rot and tender twig drying under Banda and Jhansi conditions.

The disease was recorded in all the 20 locations at Banda observed during 2002, 2003, 2004 (Three years) and 15 locations at Jhansi in 2003 and 2004 (Two years) being maximum (21.4%) at Kanwara causing fruit rot and 15.0% at Jari causing twigs drying.

The disease incidence is more during January/February as compared to September/October.

Alternaria alternata was predominantly and constantly found associated with infected fruits, twig, stem, seed collected from different places. The fungal morphology and cultural characters were studied after purification by hyphal tip and single spore culture of various isolates.

The symptoms incited by *Alternaria alternata* were critically examined and recorded on seeds, seedlings, stem, leaves and fruits.

The pathogenicity of the fungus was proved by seed infestation, soil-infestation and fruit inoculation method. Seed rot upto 63% was recorded in seed infestation method, after rotting the healthy seeds on actively grown culture and thereafter plating on the blotters. In soil infestation method, pre-emergence mortality up to 29.0% and subsequent post emergence mortality up to 11.0% due to the fungus was recorded when the sterilized soil was mixed with mycelia mat and chilli seeds were

grown. Out of four methods of fruit inoculation, pin prick method was found to be the best as the typical symptoms appeared within 7 days on intact and detached chilli leaves, semi ripe and ripe fruits.

Association of *Alternaria alternata* and other mycoflora was studied by standard blotter method and standard agar plate method in 50 chilli seed samples surface disinfected with 0.1% mercuric chloride and untreated. In all, 17 fungi were recorded which include *A. alternata*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Botrytis cinerea*, *Curvularia lunata*, *Curvularia* sp., *Chaetomium* sp., *Cladosporium oxysporum*, *Colletotrichum capsici* (= *C. dematium*), *Drechslera tetramera*, *Fusarium oxysporum*, *F. solani*, *Memnoniella* sp., *Penicillium* sp., *Rhizopus* sp. and *Alternaria* sp. Based on the maximum natural seed infection of *A. alternata*, four seed samples were selected for further studies.

Two categories of seeds, apparently healthy bold seeds and rusty brown shriveled seeds were made by naked eye examination and the seeds were tested by standard blotter method for the associated *A. alternata* which revealed the higher (30.25%) association in rusty brown seeds as compared to healthy seeds indicating the need to take care during seed process.

Cut of 12 methods used for the direct detection of Seed-borne *A. alternata* with chilli seeds, standard blotter method was the best as the maximum (32.0%) count was recorded.

Out of 10 non-synthetic and synthetic media, potato dextrose agar medium was the best. Out of four levels of temperature and eight levels of pH, 30°C and pH 6.5 was the best for sporulation and growth of the fungus. For spore germination, 22°C, 90% humidity was found to be the best. Among five concentrations of sucrose, 0.01 and 0.25% were the best where complete spore germination took place within 3 hours. Inhibitory influence of chilli seed exudation was recorded on spore germination

where 72.0% spore germination was recorded as compared to control (96.0%).

The fungus was found to be associated as externally as well as internally with chilli seeds. Seed to seedling transmission was recorded and upto 37.0% mortality was observed in test tube agar seedling symptom test and growing on test. Disintegration and discolouration of epidermis, palisade and mesophyll tissues was observed in histopathological studies of infected chilli leaves. Infection of cuticular, epidermal and hypodermal tissue was noted in stem.

Plant to seed transmission of the fungus was recorded by collecting and analysis of seeds from the inoculated fruits at different growth stages. Maximum (72.0%) recovery was recorded at yellow red stage inoculated fruits. The fungus was recorded in pericarp, septum, placenta and seeds.

Survival of the fungus could be achieved upto 120 days from infected twigs, 150 days from fruits and 210 days from the seeds stored in paper envelopes at ordinary room temperature, while in soil the viability could only be noticed up to 120 days.

Production of toxic metabolites by the fungus was recorded. Bioassay method revealed the maximum seed germination reduction (70.24%) when the seeds were soaked in culture filtrate from 15 day old culture. Influence of culture filtrate dilutions and soaking time in filtrate was studied. Inhibition in root and shoot length was recorded when exposed to culture filtrate. The root length (3.0mm) and shoot length (2.0mm) were recorded when subjected to crude filtrate as compared to 65.0mm and 34.0mm in distilled water, respectively. Seeds soaked in culture filtrate for 1, 2 and 3 hours exhibited 18.0, 24.0 and 26.0% seed germination, while seeds soaked for more than 3 hours did not germination. Influence of different temperatures on the activity of toxic material revealed that filtrate heated upto 50°C exhibited lesser (30°C)

seed germination as compared to 87.0% in control. Maximum (48.0%) increase in fruit rot was observed at 100% humidity in Pusa Jwala. Maximum (54.0%) increase in fruit rot was at 25°C. Yellow red stage was the most susceptible as compared to green-yellow, young green fruits.

Reduction in ascorbic acid content, capsaicin content and carotene content was noted in all the varieties tested after infection of the fungus being maximum in C-1 Pant, Pusa Jwala, respectively.

Fungal infection had an adverse effect on the plant height, number, length, girth and weight of the fruits as compared to healthy plants in all the varieties tested.

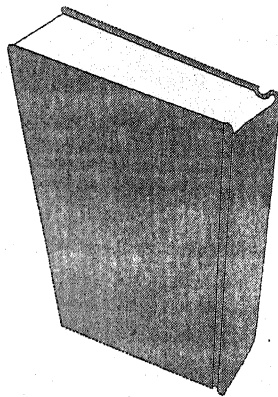
Among 21 chilli varieties evaluated against the disease Kaliyanpur Yellow, G3, SG 5, JCA 181, JCA 232, K 2, Musalwadi and LCA 206 exhibited moderate resistance exhibiting the minimum disease index (20.5) in JCA 181.

Out of eight fungicides evaluated *in vitro* by poisoned food technique, Triforine (0.15%) exhibited maximum inhibition (15.0mm) as compared to the control (86.5mm colony growth).

Seed-borne association of the fungus was drastically reduced when the chilli seeds were treated with Thiram + Captan (1:1) showing only 3.0% fungal infection as compared to 31.0% in control.

Under natural field conditions of IGFR, Jhansi and Banda, maximum disease control (81.58%) was achieved by three applications of Triforin (0.15%) starting from flowering to ripening of chilli fruit.

SUMMARY



SUMMARY

Chilli (*Capsicum annum* L.) is an indispensable condiment as well as vegetable in every household in India. No Indian dish, either vegetarian or non-vegetarian, is complete without it. Chillies are valued throughout the world for its pungency, aroma and medicinal value. Chillies are one of the major and cheapest source of Vitamin C, and A Chilli fruit and drink are used as an irritant for rheumatism or neuritis. Chilli is a unique crop among spices being the only source of capsaicin ($C_{18}H_{27}NO_3$) an alkaloid the hot active principle which is a mixture of seven closely related alkylvanillylamide named capsaicinoides. The red colour of the fruit is due to the pigment capsanthin.

In the present investigation, the fruit rot incited by *Alternaria alternata* (Fr.) Keissler has been identified a major constraint in the production of chillies. The fungus is responsible for seed rot, seedling decay, leaf spots, fruit rot and tender tip drying at different stages of crop growth.

In the present investigation, during survey of 20 locations at Banda 2002, 2003 and 2004 and 15 locations at IGFR, Jhansi during 2003 and 2004, the disease has been recorded in all the places, being maximum (21.4%) at Kanwara causing fruit rot and 15.0% at Jari, responsible for twig drying. In Jhansi out of 15 villages, maximum 23.3% fruit rot was recorded at karari village and 17.2% twig drying was recorded at Chirgaon village. Hence the disease incidence Varied at different locations.

It is interesting to note that the crop is usually grown by every farmer either for domestic purpose or for commercial purpose.

During all the 45 locations were visited and it was conclusively established that the disease is widespread, appearing every year and assuming serious and increasing proportions. It was also recorded that the disease is more prevalent during January/February as compared to September/October crop.

Alternaria alternata was predominantly and constantly found associated with infected fruits, twig, stem, seeds showing the symptoms. No critical variation in the isolates were observed. The fungus was purified by fungal hyphal tip method and subsequently by single spore culture method. The fungus was identified with the help of characters mentioned by Bllis (1971) and subramaniam (1971). The fungal morphology, colony Characters and measurements of hyphae and conidia were taken for documentation.

In the present investigation, symptoms incited by the fungus on seeds, seedlings, stem, leaves and fruits were recorded.

The virulence of the isollted *Alternaria alternata* was tested by three methods, viz. seed-infestation, soil infestation and fruit inoculation. Upto 63.0% rotting of chilli seeds was recorded when healthy chilli seeds were infested with actively grown pure culture. Artificially injured seeds had greater seed rot. In soil infestation method, pre-emergence mortality upto 29.0% and subsequent post-emergence mortality upto 11.0% due to the fungus was recorded where the mycelial mat was mixed with sterilized soils and incubated in pot house. In fruit inoculation test, semi ripe and ripe fruits of Pusa Jwala were inoculated by four methods. Pin prick method was found to be the best where symptoms appeared on chilli leaves, semi ripe and ripe fruits in intact and detached condition exhibited infection within 7 to 9 days. Ripe fruits were more vulnerable to infection and higher association of *Alternaria alternata* was observed with ripe fruits.

Association of *Alternaria alternata* and other mycoflora with chilli seeds was observed by Standard blotter and Standard Agar Plate Method. In all, 50 seed samples were collected from 20 locations during visit, 11 from IVRI, Varanasi and 19 varieties from the Department of Horticulture, CSAUAT, Kanpur. The association of the mycoflora was observed on untreated and treated chilli seeds with 0.1% mercuric chloride. In all, 17 fungi were observed associated with chilli seeds which include *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *A. fumigates*, *Botrytis cinerea*, *Curvularia lunata*, *Curvularia sp.*, *Chaetomium sp.*, *Cladosporium oxysporum*, *Colletotrichum capsici* (= *C. dematium*), *Drechslera tetramera*, *Fusarium oxysporum*, *F. solani*, *Memnoniella sp.*, *Penicillium sp.*, *Rhizopus sp.* and *Alternaria sp.*

Based on the maximum natural infection of *A. alternata* four seed samples from Jhansi (Sample No. 21 having 28.0% natural infection), Hamirpur (Sample No. 24 having 28.0% association), Mahoba (Sample No. 27 having 30.0% association) and Banda (Sample No. 30 having 32.0% association) were selected and used for further studied throughout the investigation.

By naked eye examination, seeds of chilli were grouped into two, apparently healthy bold seed and second group having rusty brown shriveled seeds. The seeds were tested by Standard blotter method and the relation of apparent seed quality and associated mycoflora was determined. Shriveled seeds had 30.25% association of *A. alternata* as compared to 28.5% in bold seeds. Healthy apparently bold seeds had lesser number of mycoflora and higher germination percentage indicating the need to process the seeds carefully. On the floor and removing the uneven, shriveled rusty seeds.

For the direct detection of *Alternaria alternata* under stereoscopic binocular, four incubation methods, Standard blotter method, Standard

agar plate method, 2, 4-D method and Deep Freeze method were used using the four selected seed samples. Previously selected and tested by Standard blotter method. Modification of these four methods were also tried, by Changing pH of water used for soaking the blotter's by changing agar medium, by seed and blotter dip in 2, 4-D solution and by soaking blotter and seed in antibiotic solution. In all, the detection of the fungus associated with seed was tried in 12 days on four seed samples. Comparative efficacy of various methods revealed that the Standard blotter method was the best in which maximum 32.0% association was found.

Seed-borne nature of the fungus was established in the chilli seeds by various tests.

Influence of different media, temperature, pH and other factors on the growth, sporulation and spore germination of *A. alternata* was studied. Out of ten synthetic and non-synthetic media used, maximum growth and sporulation was recorded on Potato dextrose agar medium. Four levels of temperature and eight levels of pH were tried and it was recorded that the fungus grew best on 30°C with maximum growth and sporulation at pH 6.5. In the present investigation, it was observed that the fungal spores started germinating within 4:30 hours at 22°C. Unipolar, bipolar and septal germination was recorded. Spores germinated at 90.0% humidity within 6:00 hours while at 25.0% no germination was recorded. Among five concentrations of sucrose 0.01 and 0.25% were the best where complete germination of spores took place within 3 hours only. The carbohydrate was used for exogenous supply. Inhibitory influence of chilli seed exudation was recorded on spore germination where 72.0% germination was recorded as compared to 96% in control.

Seed-borne nature of the fungus was established by testing seed samples. Transmission of the fungus from seed to seedlings was

confirmed by water agar seedling symptom test and growing on test. Total 37.0% mortality was recorded due to *A. alternata*. In pots, 27.0% seed rot and 9.0% seedling infection was confirmed.

Site of infection was confirmed by component seed plating, histopathological studies and plating pretreated and untreated seeds on blotters. Plant to seed transmission of the fungus was confirmed by inoculating chilli fruits and subsequent analysis of fruit parts of the harvest. Fruit inoculated at yellow red stage exhibited 72.0% infection, green yellow (67.0%) and at green stage 40.0%. Establishment of the infection was confirmed in pericarp, septum, seed, placenta by plating on PDA. Association of the fungus was confirmed from all the parts of collected plants except roots. The histopathological studies revealed the colonisation of epidermis, palisade and mesophyll tissues of leaves. Infected tissues turned brown and collapsed and the content became coarsely granular. In stem, epidermal and subepidermal portions were found deformed and disintegrated. Periodical isolation by the plating of portions of twigs and fruits stored in paper envelopes could be made upto 120 days from infected twig, 150 days from fruits and 210 days from seeds indicating the survival of *A. alternata* upto about 7 months. In soils, isolations from the plant part mixed in the soil and kept at room temperature could be made upto only 120 days.

Production of toxic metabolites by the fungus was confirmed by bioassay method. Maximum per cent reduction in seed germination was 70.24% when the seeds were soaked in culture filtrate from 15 day old culture. The influence of dilution of culture filtrate indicated that in crude culture filtrate the seed germination was only 20.0% as compared to 84.0% in control filtrate obtained from uninoculated medium and 86.0% from distilled water. With the increase in dilution the improvement in seed germination was noticed. Elongation of root and shoot was also

adversely affected due to the metabolites produced by the fungus. The influence on root length (3.0mm), shoot length (2.0mm) was recorded when subjected to crude filtrate as compared to 65.0mm and 34.0mm in distilled water, respectively. It was also recorded that increase in soaking time in filtrate had adverse effect on germination. Seeds soaked in the culture filtrate for more than 3 hours did not germinate at all. Seeds soaked in culture filtrate for 1, 2, 3 hours exhibited 18.0, 24.0 and 26.0% seed germination as compared to 63.0 to 73.0% when the seeds were soaked in either distilled water or uninoculated Richard's medium filtrate. Influence of temperature on the activity of toxic metabolites was observed and it was concluded that when the filtrate was heated upto 50°C, exhibited comparatively lesser seed germination (30.0%) as compared to distilled water treated seeds (87.0% seed germination).

Factors affecting disease development were studied. Influence of temperature, humidity and age of chilli fruits on the development of fruit rot incited by the fungus was observed. Maximum (48.0%) increase in fruit rot was observed in Pusa Jwala at 100% humidity, similarly maximum (54.0%) increase in fruit rot was observed at 25°C in Pusa Jwala. At lower temperature (10° and 15°C) development rate was quite low. Susceptibility of the fruits of all the four varieties increased with increase in age. Yellow red stage was the most susceptible as compared to green yellow stage. Young green fruits were least susceptible while red ripe stage was most vulnerable.

The fungal infection brought great shifts in the nutritive value of the chilli fruits. Reduction in ascorbic acid, capsaicin content and carotene was observed in the present investigation in all the four varieties. Maximum (41.85%) reduction in ascorbic acid content was noticed in C-1 Pant, followed by Pandurna (36.45%). Maximum reduction in carotene content was 53.89% in Pusa Jwala, followed by 44.51% in Jawahar

Mirch-218. It was interesting to note that with the increased age the contents were also decreased, Maximum (49.65%) reduction in capsaicin content was noted in Pusa Jwala while minimum (21.57%) in Pandurna. The capsaicin reduction ranged 21.57 to 49.65% in all the four chilli varieties.

Influence of fungal infection also had an adverse effect on the plant height, number of fruits, length and girth of fruits and weight of the fruits as compared to healthy plants. Maximum (33.4%) reduction in plant height was noticed in C-1 Pant at 130 days. In Pusa Jwala least number of chilli fruits were observed being the maximum reduction of 39.9%. Reduction in fruit length was maximum in Pusa Jwala (30.56%) and in fruit girth in Pandurna (22.22%).

Attempts for the management of disease were made by evaluation of germplasm and fungicides under laboratory condition, use of seed dresser and by foliar application.

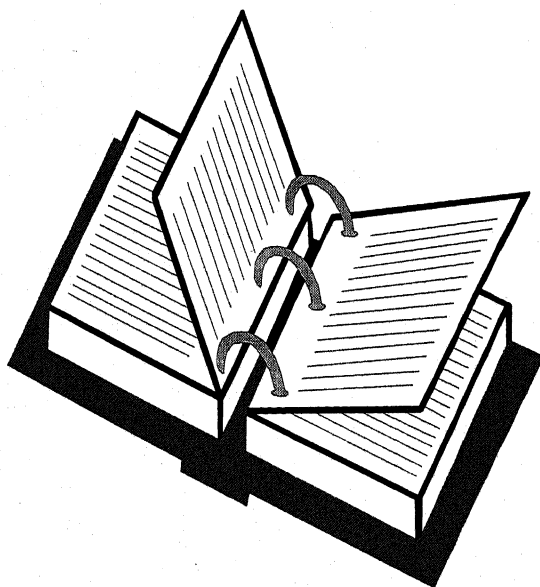
Twenty-one chilli varieties were evaluated against the disease under natural field conditions at Banda however, none of the variety exhibited desired resistance. Kaliyanpur Yellow, G 3, SG 5, JCA 181, JCA 232, K 2, Musalwadi and LCA 206 showed the moderate resistance. Minimum disease index (20.5) was recorded in JCA 181, while maximum (24.5) in Musalwadi.

Under laboratory condition, out of eight fungicides evaluated by poisoned food technique, Triforine (0.15% conc.) was the most inhibitory exhibiting only 15.5 mm colony growth as compared to control 86.5mm.

Seed-borne inoculums was drastically reduced when the chilli seeds were treated with Thiram + Captan (1:1) showing pared to 31.0% in control when tested by Standard blotter method. Seed dressing with fungicide also improved the germination.

Under field conditions of IGFRI, Jhansi and Banda reduction in fruit rot disease was recorded by foliar application of fungicides. Out of six fungicides tested, maximum (81.58%) disease control was achieved in Triforine (0.15%) treated plots applied three times starting from flowering to ripening at Banda. Similar trend was recorded at IGFRI, Jhansi. Triforin and Indofil M-45 applied as foliar spray were found to be better in controlling disease.

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